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(54) Title: RHESUS CARCINO EMBRYONIC ANTIGEN, NUCLEOTIDES ENCODING SAME, AND USES THEREOF

(57) Abstract: DNAs encoding rhesus monkey carcinoembryonic antigen (rhCEA) have been isolated, cloned and sequenced. The gene encoding CEA is commonly associated with the development of human carcinomas. The present invention provides compositions and methods to elicit or enhance immunity to the protein product expressed by the CEA tumor-associated antigen, wherein aberrant CEA expression is associated with a carcinoma or its development. This invention specifically provides adenoviral vector constructs carrying rhCEA and discloses their use in vaccines and pharmaceutical compositions for preventing and treating cancer.

TITLE OF THE INVENTION

RHESUS CARCINO EMBRYONIC ANTIGEN, NUCLEOTIDES ENCODING SAME, AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates generally to the therapy of cancer. More specifically, the present invention relates to the rhesus monkey homologue of the tumor associated polypeptide carcinoembryonic antigen, herein designated rhCEA, to isolated nucleic acid molecules which encode this protein, and to recombinant vectors and hosts comprising DNA encoding this protein. This invention also relates to adenoviral vector constructs carrying rhCEA and to their use in vaccines and pharmaceutical compositions for preventing and treating cancer.

BACKGROUND OF THE INVENTION

The immunoglobulin superfamily (IgSF) consists of numerous genes that code for proteins with diverse functions, one of which is intercellular adhesion. IgSF proteins contain at least one Ig-related domain that is important for maintaining proper intermolecular binding interactions. Because such interactions are necessary to the diverse biological functions of the IgSF members, disruption or aberrant expression of many IgSF adhesion molecules has been correlated with many human diseases.

The carcinoembryonic antigen (CEA) belongs to a subfamily of the Ig superfamily consisting of cell surface glycoproteins. Members of the CEA subfamily are known as CEA-related cell adhesion molecules (CEACAMs). In recent scientific literature, the CEA gene has been renamed CEACAM5, although the nomenclature for the protein remains CEA. Functionally, CEACAMs have been shown to act as both homotypic and heterotypic intercellular adhesion molecules (Benchimol et al., *Cell* 57: 327-334 (1989)). In addition to cell adhesion, CEA inhibits cell death resulting from detachment of cells from the extracellular matrix and can contribute to cellular transformation associated with certain proto-oncogenes such as *Bcl2* and *C-Myc* (*see* Berinstein, *J. Clin Oncol.* 20(8): 2197-2207 (2002)).

Normal expression of CEA has been detected during fetal development and in adult colonic mucosa. CEA overexpression was first detected in human colon tumors over thirty years ago (Gold and Freedman, *J. Exp. Med.* 121:439-462 (1965)) and has since been found in nearly all colorectal tumors.

Additionally, CEA overexpression is detectable in a high percentage of adenocarcinomas of the pancreas, breast and lung. Because of the prevalence of CEA expression in these tumor types, CEA is widely used clinically in the management and prognosis of these cancers.

The correlation between CEA expression and metastatic growth has also led to its identification as a target for molecular and immunological intervention for colorectal cancer treatment. One therapeutic approach targeting CEA is the use of anti-CEA antibodies (*see* Chester et al., *Cancer Chemother. Pharmacol.* 46 (Suppl): S8-S12 (2000)), while another is to activate the immune system to attack CEA-expressing tumors using CEA-based vaccines (for review, *see* Berinstein, *supra*).

Sequences coding for human CEA have been cloned and characterized (U.S. Patent No. 5,274,087; U.S. Patent No 5,571,710; and U.S. Patent No 5,843,761. See also Beauchemin et al., *Mol. Cell. Biol.* 7:3221-3230 (1987); Zimmerman et al., *Proc. Natl. Acad. Sci. USA* 84:920-924 (1987); Thompson et al. *Proc. Natl. Acad. Sci. USA* 84(9):2965-69 (1987)). Despite the isolation and identification of these CEA genes, it would be desirable to identify additional mammalian genes encoding CEA to allow for the development of a cancer vaccine which is efficacious and not hindered by self-tolerance.

SUMMARY OF THE INVENTION

The present invention relates to isolated or purified nucleic acid molecules (polynucleotides) comprising a sequence of nucleotides that encode a novel rhesus monkey carcino embryonic antigen (hereinafter rhCEA) as set forth in SEQ ID NO:2 and SEQ ID NO:18. The DNA molecules disclosed herein may be transfected into a host cell of choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional rhCEA protein (SEQ ID NO:2 and SEQ ID NO:18).

The present invention further relates to an isolated nucleic acid molecule which encodes mRNA that expresses a novel rhesus monkey CEA protein; this DNA molecule comprising the nucleotide sequence disclosed herein as SEQ ID NO:1. Nucleotide sequences coding for rhesus CEA are herein designated rhCEACAM5. A preferred aspect of this portion of the present invention is disclosed in FIGURE 1A, which shows a DNA molecule (SEQ ID NO:1) that encodes a novel rhCEA protein (SEQ ID NO:2).

Another aspect of this invention is an isolated nucleic acid molecule which encodes a novel rhesus monkey CEA protein (SEQ ID NO:18), said nucleic acid molecule comprising a sequence of nucleotides as shown in FIGURE 1B and as set forth in SEQ ID NO:5.

The present invention also relates to recombinant vectors and recombinant host cells, both prokaryotic and eukaryotic, which contain the nucleic acid molecules disclosed throughout this specification.

The present invention further relates to a process for expressing a rhesus monkey CEA protein in a recombinant host cell, comprising: (a) introducing a vector comprising a nucleic acid as set forth in SEQ ID NO:1 or SEQ ID NO:5 into a suitable host cell; and, (b) culturing the host cell under conditions which allow expression of said rhesus monkey CEA protein.

A preferred aspect of the present invention is a substantially purified form of a rhesus monkey CEA protein which consists of the amino acid sequence disclosed in FIGURE 2A (SEQ ID NO:2).

Another preferred aspect of the present invention is a substantially purified form of a rhesus monkey CEA protein which consists of the amino acid sequence disclosed in FIGURE 2B (SEQ ID NO:18).

Another preferred aspect of the present invention relates to a substantially purified, fully processed (including proteolytic processing, glycosylation and/or phosphorylation), mature rhCEA protein obtained from a recombinant host cell containing a DNA expression vector comprising nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:5, which express the rhCEA protein. It is especially preferred that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line.

Yet another aspect of this invention is a method of preventing or treating cancer comprising administering to a mammal a vaccine vector comprising an isolated nucleic acid molecule, the isolated nucleic acid molecule comprising a sequence of nucleotides that encodes a rhesus monkey carcinoembryonic antigen (rhCEA) protein as set forth in SEQ ID NO:2 or SEQ ID NO:18.

The present invention further relates to an adenovirus vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising: (a) a

polynucleotide encoding a rhesus monkey CEA protein; and (b) a promoter operably linked to the polynucleotide.

The present invention also relates to a vaccine plasmid comprising a plasmid portion and an expression cassette portion, the expression cassette portion comprising: (a) a polynucleotide encoding a rhesus monkey CEA protein; and (b) a promoter operably linked to the polynucleotide.

Another aspect of the present invention is a method of protecting or a mammal from cancer or treating a mammal suffering from cancer comprising: (a) introducing into the mammal a first vector comprising: i) a polynucleotide encoding a rhesus monkey CEA protein; and ii) a promoter operably linked to the polynucleotide; (b) allowing a predetermined amount of time to pass; and (c) introducing into the mammal a second vector comprising: i) a polynucleotide encoding a rhesus monkey CEA protein; and ii) a promoter operably linked to the polynucleotide.

As used throughout the specification and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise.

As used throughout the specification and appended claims, the following definitions and abbreviations apply:

The term "promoter" refers to a recognition site on a DNA strand to which the RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences termed "enhancers" or inhibiting sequences termed "silencers".

The term "cassette" refers to the sequence of the present invention that contains the nucleic acid sequence which is to be expressed. The cassette is similar in concept to a cassette tape; each cassette has its own sequence. Thus by interchanging the cassette, the vector will express a different sequence. Because of the restriction sites at the 5' and 3' ends, the cassette can be easily inserted, removed or replaced with another cassette.

The term "vector" refers to some means by which DNA fragments can be introduced into a host organism or host tissue. There are various types of vectors including plasmid, virus (including adenovirus), bacteriophages and cosmids.

The term "first generation," as used in reference to adenoviral vectors, describes said adenoviral vectors that are replication-defective. First generation adenovirus vectors typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region.

The designation "pV1J-rhCEA" refers to a plasmid construct disclosed herein comprising the human CMV immediate-early (IE) promoter with intron A, a full-length rhesus CEA gene, bovine growth hormone-derived polyadenylation and transcriptional termination sequences, and a minimal pUC backbone.

The designations "pMRK-Ad5-rhCEA" and "MRK-rhCEA" refer to a construct, disclosed herein, which comprises an Ad5 adenoviral genome deleted of the E1 and E3 regions. In this plasmid, the E1 region is replaced by a rhesus CEA gene in an E1 parallel orientation under the control of a human CMV promoter without intron A, followed by a bovine growth hormone polyadenylation signal.

The designation "pBS-rhCEA" refers to a construct disclosed herein comprising the pBluescriptII KS (+) plasmid and a full-length rhCEA gene.

The term "effective amount" means sufficient vaccine composition is introduced to produce the adequate levels of the polypeptide, so that an immune response results. One skilled in the art recognizes that this level may vary.

"Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. As used interchangeably, the terms "substantially free from other nucleic acids," "substantially purified," "isolated nucleic acid" or "purified nucleic acid" also refer to DNA molecules which comprise a coding region for a rhesus CEA protein that has been purified away from other cellular components. Thus, a rhesus CEA DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-rhesus CEA nucleic acids. Whether a given rhesus CEA DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

"Substantially free from other proteins" or "substantially purified" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a rhesus monkey CEA protein preparation that is

substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-rhesus monkey CEA proteins. Whether a given rhesus monkey CEA protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

As used interchangeably, the terms "substantially free from other proteins" or "substantially purified," or "isolated rhesus monkey CEA protein" or "purified rhesus monkey CEA protein" also refer to rhesus monkey CEA protein that has been isolated from a natural source. Use of the term "isolated" or "purified" indicates that rhesus monkey CEA protein has been removed from its normal cellular environment. Thus, an isolated rhesus monkey CEA protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated rhesus monkey CEA protein is the only protein present, but instead means that an isolated rhCEA protein is substantially free of other proteins and non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the rhCEA protein *in vivo*. Thus, a rhesus monkey CEA protein that is recombinantly expressed in a prokaryotic or eukaryotic cell and substantially purified from this host cell which does not naturally (*i.e.*, without intervention) express this rhCEA protein is of course "isolated rhesus monkey CEA protein" under any circumstances referred to herein. As noted above, a rhCEA protein preparation that is an isolated or purified rhCEA protein will be substantially free from other proteins and will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-rhesus monkey CEA proteins.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

"rhCEA" refers to a rhesus monkey carcinoembryonic antigen.

The term "mammalian" refers to any mammal, including a human being.

The abbreviation "Ag" refers to an antigen.

The abbreviations "Ab" and "mAb" refer to an antibody and a monoclonal antibody, respectively.

The abbreviation "ORF" refers to the open reading frame of a gene.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows nucleotide sequences of the rhesus monkey CEA cDNA molecules, as set forth in SEQ ID NO:1 (Panel A) and SEQ ID NO:5 (Panel B). *See* EXAMPLE 2.

FIGURE 2 shows the predicted amino acid sequences of the first rhesus monkey CEA protein, as set forth in SEQ ID NO:2 (Panel A) and the second rhesus monkey CEA protein, as set forth in SEQ ID NO:18 (Panel B). The two amino acid differences between the first and the second rhesus CEA proteins are bold and underlined in Panel B.

FIGURE 3 shows an alignment of the 5' untranslated region of human CEACAM family members. Sequences shown were compared and used to design degenerate primers as described in EXAMPLE 2. Nucleotides that are the same as the corresponding nucleotide in other CEACAM family members are highlighted. Dashes indicate that spaces were added to facilitate alignment of the sequences. Nucleotide number of each cDNA sequence, as disclosed in GenBank, is shown in parentheses.

FIGURE 4 shows the expression of the rhesus CEA protein. HeLa cells were transfected with phagemids obtained by screening the lambda-CEA library and a western blot was performed using a rabbit polyclonal antibody vs. human CEA protein. Expression of 2 clones out of 15 is shown.

FIGURE 5 shows a schematic representation of the rhesus CEA coding region. Internal repetitions are indicated and restriction sites for gene fragmentation and sequence are reported.

FIGURE 6 shows an alignment of the human (SEQ ID NO:6) and rhesus (SEQ ID NO:1) CEACAM-5 nucleotide sequences. Nucleotides that are different between the two CEACAM-5 sequences are shown in bold.

FIGURE 7 shows an alignment of the human (SEQ ID NO:7) and rhesus (SEQ ID NO:2) CEACAM-5 open reading frames. Amino acids that are different between the two CEACAM-5 sequences are shown in bold.

FIGURE 8 shows the humoral response against human CEA in CEA transgenic mice. The average antibody titer is given for two groups of mice: one immunized with rhesus CEA and one immunized with human CEA (EXAMPLE 7).

FIGURE 9 shows the cell mediated immune response against human CEA in CEA transgenic mice. CEA transgenic mice were vaccinated either with hCEA expressing vectors or with rhCEA expressing vectors (EXAMPLE 9).

FIGURE 10 shows the cell mediated immune response against rhesus CEA peptides in CEA transgenic mice immunized with rhesus or human CEA.

DETAILED DESCRIPTION OF THE INVENTION

The gene encoding the carcinoembryonic antigen (CEA) is commonly associated with the development of adenocarcinomas. The present invention relates to compositions and methods to elicit or enhance immunity to the protein product expressed by the CEA tumor-associated antigen, wherein aberrant CEA expression is associated with the carcinoma or its development. Association of aberrant CEA expression with a carcinoma does not require that the CEA protein be expressed in tumor tissue at all timepoints of its development, as abnormal CEA expression may be present at tumor initiation and not be detectable late into tumor progression or vice-versa.

To this end, polynucleotides encoding rhesus monkey carcinoembryonic antigen (rhCEA) are provided. The molecules of the present invention may be used in a recombinant adenovirus or plasmid-based vaccine to provide effective immunoprophylaxis against adenocarcinomas through cell-mediated immunity. When directly introduced into a vertebrate *in vivo*, the invention polynucleotides induce the expression of encoded proteins within the animal, including mammals such as primates, dogs and humans.

The present invention relates to an isolated nucleic acid molecule (polynucleotide) comprising a sequence of nucleotides which encodes mRNA that expresses a novel rhCEA protein as set forth in SEQ ID NO:2 or SEQ ID NO:18. The nucleic acid molecules of the present invention are substantially free from other nucleic acids.

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecules of the present invention may also include a ribonucleic acid molecule (RNA). For most cloning purposes, DNA is a preferred nucleic acid.

A preferred DNA molecule of the present invention comprises the nucleotide sequence disclosed herein as SEQ ID NO:1, shown in FIGURE 1A, which encodes the rhesus CEA protein shown in FIGURE 2A and set forth as SEQ ID NO:2.

Another preferred DNA molecule of the present invention comprises the nucleotide sequence disclosed herein as SEQ ID NO:5 (hereinafter "second rhCEA" DNA sequence), shown in FIGURE 1B, which encodes the rhesus CEA protein shown in FIGURE 2B and set forth as SEQ ID NO:18. These rhCEA nucleic acid molecules were identified through RT-PCR as described in detail in EXAMPLE 2. The second rhCEA DNA sequence (SEQ ID NO:5) differs from the first by two nucleotides and was cloned from colon tissue from a different rhesus monkey. This DNA sequence codes for a rhesus CEA protein that differs from the first rhesus CEA protein by two amino acids.

The isolated cDNA clones, associated vectors, hosts, recombinant subcellular fractions and membranes, and the expressed and mature forms of rhCEA are useful for the development of a cancer vaccine.

The present invention also includes biologically active fragments or mutants of SEQ ID NOs:1 or 5, which encode mRNA expressing novel rhCEA proteins. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the pharmacological properties of the rhCEA protein, including but not limited to the rhCEA protein as set forth in SEQ ID NO:2 or SEQ ID NO:18. Any such polynucleotide includes but is not necessarily limited to: nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations. The mutations of the present invention encode mRNA molecules that express a functional rhCEA protein in a eukaryotic cell so as to be useful in cancer vaccine development.

This invention also relates to synthetic DNA that encodes the rhCEA protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ ID NO:1 and SEQ ID NO:5, but still encodes the rhCEA protein as set forth in SEQ ID NO:2 or SEQ ID NO:18. Such synthetic DNAs are intended to be within the scope of the present invention.

Therefore, the present invention discloses codon redundancy that may result in numerous DNA molecules expressing an identical protein. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations either in the DNA sequence or the translated protein that do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in the functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide that has properties that are different than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but are not limited to changes in the affinity of an enzyme for a substrate or receptor for a ligand.

Included in the present invention are DNA sequences that hybridize to SEQ ID NO:1 or SEQ ID NO:5 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography. Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook et al., *Molecular Cloning: A*

Laboratory Manual; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989, which is hereby incorporated by reference. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

A preferred aspect of the present invention is a substantially purified form of a rhesus monkey CEA protein which comprises a sequence of amino acids as disclosed in FIGURE 2A (SEQ ID NO:2).

Another preferred aspect of the present invention is a substantially purified form of a rhesus monkey CEA protein which comprises a sequence of amino acids as disclosed in FIGURE 2B (SEQ ID NO:18).

This invention also relates to various functional domains of rhCEA and to hybrid molecules comprising at least one of these sequences. The CEA protein comprises an amino-terminal domain with a processed leader sequence and a hydrophobic carboxy-terminal domain. CEA also comprises three Ig-like internal domains. Subdomains of the N-terminal domain were shown by Taheri et al. (*J. Biol. Chem.* 275(35): 26935-26943 (2000)) to be required for CEA's intercellular adhesion function.

The present invention also includes biologically active fragments and/or mutants of a rhCEA protein, comprising the amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO:18, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for cancer vaccine development.

The rhesus monkey CEA proteins of the present invention may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The present invention also relates to rhCEA fusion constructs, including but not limited to fusion constructs which express a portion of the rhesus CEA protein linked to various markers, including but in no way limited to GFP (Green fluorescent protein), the MYC epitope, GST, and Fc. Any such fusion construct may be expressed in the cell line of interest and used to screen for

modulators of the rhesus CEA protein disclosed herein. Also contemplated are fusion constructs that are constructed to enhance the immune response to rhesus CEA including, but not limited to: DOM and hsp70.

The present invention further relates to recombinant vectors that comprise the substantially purified nucleic acid molecules disclosed throughout this specification. These vectors may be comprised of DNA or RNA. For most cloning purposes, DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a rhCEA protein. It is well within the purview of the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

An expression vector containing DNA encoding a rhCEA protein may be used for expression of rhCEA in a recombinant host cell. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Also, a variety of bacterial expression vectors may be used to express recombinant rhCEA in bacterial cells if desired. In addition, a variety of fungal cell expression vectors may be used to express recombinant rhCEA in fungal cells. Further, a variety of insect cell expression vectors may be used to express recombinant protein in insect cells.

The present invention also relates to host cells transformed or transfected with vectors comprising the nucleic acid molecules of the present invention. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey and rodent origin; and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Such recombinant host cells can be cultured under suitable conditions to produce rhCEA or a biologically equivalent form.

As noted above, an expression vector containing DNA encoding a rhCEA protein may be used for expression of rhCEA in a recombinant host cell. Therefore, another aspect of this invention is a process for expressing a rhesus monkey CEA protein in a recombinant host cell, comprising: (a) introducing a vector comprising a nucleic acid as set forth in SEQ ID NO:1 or SEQ ID NO:5 into a suitable host cell; and, (b) culturing the host cell under conditions which allow expression of said rhesus monkey CEA protein.

Following expression of rhCEA in a host cell, rhCEA protein may be recovered to provide rhCEA protein in active form. Several rhCEA protein purification procedures are available and suitable for use. Recombinant rhCEA protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant rhCEA protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length rhCEA protein, or polypeptide fragments of rhCEA protein.

The nucleic acids of the present invention may be assembled into an expression cassette which comprises sequences designed to provide for efficient expression of the protein in a human cell. The cassette preferably contains the full-length rhCEA gene, with related transcriptional and translations control sequences operatively linked to it, such as a promoter, and termination sequences. In a preferred embodiment, the promoter is the cytomegalovirus promoter without the intron A sequence (CMV), although those skilled in the art will recognize that any of a number of other known promoters such as the strong immunoglobulin, or other eukaryotic gene promoters may be used. A preferred transcriptional terminator is the bovine growth hormone terminator, although other known transcriptional terminators may also be used. The combination of CMV-BGH terminator is particularly preferred.

In accordance with this invention, the rhesus CEA expression cassette is inserted into a vector. The vector is preferably an adenoviral vector, although linear DNA linked to a promoter, or other vectors, such as adeno-associated virus or a modified vaccinia virus, retroviral or lentiviral vector may also be used.

If the vector chosen is an adenovirus, it is preferred that the vector be a so-called first-generation adenoviral vector. These adenoviral vectors are characterized by having a non-functional E1 gene region, and preferably a deleted adenoviral E1 gene region. In some embodiments, the expression cassette is inserted in the position where the adenoviral E1 gene is normally located. In addition, these vectors optionally have a non-functional or deleted E3 region. It is preferred that the adenovirus genome used be deleted of both the E1 and E3 regions (ΔE1ΔE3). The

adenoviruses can be multiplied in known cell lines which express the viral E1 gene, such as 293 cells, or PERC.6 cells, or in cell lines derived from 293 or PERC.6 cell which are transiently or stablily transformed to express an extra protein. For examples, when using constructs that have a controlled gene expression, such as a tetracycline regulatable promoter system, the cell line may express components involved in the regulatory system. One example of such a cell line is T-Rex-293; others are known in the art.

For convenience in manipulating the adenoviral vector, the adenovirus may be in a shuttle plasmid form. This invention is also directed to a shuttle plasmid vector which comprises a plasmid portion and an adenovirus portion, the adenovirus portion comprising an adenoviral genome which has a deleted E1 and optional E3 deletion, and has an inserted expression cassette comprising rhesus CEA. In preferred embodiments, there is a restriction site flanking the adenoviral portion of the plasmid so that the adenoviral vector can easily be removed. The shuttle plasmid may be replicated in prokaryotic cells or eukaryotic cells.

In a preferred embodiment of the invention, the expression cassette is inserted into the pMRKAd5-HV0 adenovirus plasmid (*See* Emini et al., WO 02/22080, which is hereby incorporated by reference). This plasmid comprises an Ad5 adenoviral genome deleted of the E1 and E3 regions. The design of the pMRKAd5-HV0 plasmid was improved over prior adenovectors by extending the 5' cis-acting packaging region further into the E1 gene to incorporate elements found to be important in optimizing viral packaging, resulting in enhanced virus amplification. Advantageously, this enhanced adenoviral vector is capable of maintaining genetic stability following high passage propagation.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the adenoviruses, shuttle plasmids, and DNA immunogens of this invention.

The vectors described above may be used in immunogenic compositions and vaccines for preventing the development of adenocarcinomas associated with aberrant CEA expression and/or for treating existing cancers. To this end, one aspect of the instant invention is a method of preventing or treating cancer comprising administering to a mammal a vaccine vector comprising an isolated nucleic acid molecule, the isolated nucleic acid molecule comprising a sequence of

nucleotides that encodes a rhesus monkey CEA protein as set forth in SEQ ID NO:2 or SEQ ID NO:18.

In accordance with the method described above, the vaccine vector may be administered for the treatment or prevention of cancer in any mammal. In a preferred embodiment of the invention, the mammal is a human.

Further, one of skill in the art may choose any type of vector for use in the treatment and prevention method described. Preferably, the vector is an adenovirus vector or a plasmid vector. In a preferred embodiment of the invention, the vector is an adenoviral vector comprising an adenoviral genome with a deletion in the adenovirus E1 region, and an insert in the adenovirus E1 region, wherein the insert comprises an expression cassette comprising: (a) a polynucleotide encoding a rhesus monkey CEA protein; and (b) a promoter operably linked to the polynucleotide.

The instant invention further relates to an adenovirus vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising: (a) a polynucleotide encoding a rhesus monkey CEA protein; and (b) a promoter operably linked to the polynucleotide.

In a preferred embodiment of this aspect of the invention, the adenovirus vector is an Ad 5 vector.

In another preferred embodiment of the invention, the adenovirus vector is an Ad 6 vector.

In another aspect, the invention relates to a vaccine plasmid comprising a plasmid portion and an expression cassette portion, the expression cassette portion comprising: (a) a polynucleotide encoding a rhesus monkey CEA protein; and (b) a promoter operably linked to the polynucleotide.

In some embodiments of this invention, the recombinant adenovirus vaccines disclosed herein are used in various prime/boost combinations with a plasmid-based polynucleotide vaccine in order to induce an enhanced immune response. In this case, the two vectors are administered in a "prime and boost" regimen. For example the first type of vector is administered, then after a predetermined amount of time, for example, 1 month, 2 months, six months, or other appropriate interval, a second type of vector is administered. Preferably the vectors carry expression cassettes encoding the same polynucleotide or combination of

polynucleotides. In the embodiment where a plasmid DNA is also used, it is preferred that the vector contain one or more promoters recognized by mammalian or insect cells. In a preferred embodiment, the plasmid would contain a strong promoter such as, but not limited to, the CMV promoter. The rhesus CEA gene or other gene to be expressed would be linked to such a promoter. An example of such a plasmid would be the mammalian expression plasmid V1Jns as described (J. Shiver *et. al.* in *DNA Vaccines*, M. Liu et al. eds., N.Y. Acad. Sci., N.Y., 772:198-208 (1996), which is herein incorporated by reference).

As stated above, an adenoviral vector vaccine and a plasmid vaccine may be administered to a vertebrate as part of a single therapeutic regime to induce an immune response. To this end, the present invention relates to a method of protecting a mammal from cancer comprising: (a) introducing into the mammal a first vector comprising: i) a polynucleotide encoding a rhesus monkey CEA protein; and ii) a promoter operably linked to the polynucleotide; (b) allowing a predetermined amount of time to pass; and (c) introducing into the mammal a second vector comprising: i) a polynucleotide encoding a rhesus monkey CEA protein; and ii) a promoter operably linked to the polynucleotide.

In one embodiment of the method of protection described above, the first vector is a plasmid and the second vector is an adenovirus vector. In an alternative embodiment, the first vector is an adenovirus vector and the second vector is a plasmid.

The instant invention further relates to a method of treating a mammal suffering from an adenocarcinoma comprising: (a) introducing into the mammal a first vector comprising: i) a polynucleotide encoding a rhesus monkey CEA protein; and ii) a promoter operably linked to the polynucleotide; (b) allowing a predetermined amount of time to pass; and (c) introducing into the mammal a second vector comprising: i) a polynucleotide encoding a rhesus monkey CEA protein; and ii) a promoter operably linked to the polynucleotide.

In one embodiment of the method of treatment described above, the first vector is a plasmid and the second vector is an adenovirus vector. In an alternative embodiment, the first vector is an adenovirus vector and the second vector is a plasmid.

The amount of expressible DNA or transcribed RNA to be introduced into a vaccine recipient will depend partially on the strength of the promoters used and on the immunogenicity of the expressed gene product. In general, an

immunologically or prophylactically effective dose of about 1 ng to 100 mg, and preferably about 10 μg to 300 μg of a plasmid vaccine vector is administered directly into muscle tissue. An effective dose for recombinant adenovirus is approximately $10^6 - 10^{12}$ particles and preferably about 10^7—10^{11}particles. Subcutaneous injection, intradermal introduction, impression though the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations may be provided. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration with adjuvants such as interleukin 12 protein, concurrently with or subsequent to parenteral introduction of the vaccine of this invention is also advantageous.

The vaccine vectors of this invention may be naked, i.e., unassociated with any proteins, adjuvants or other agents which impact on the recipient's immune system. In this case, it is desirable for the vaccine vectors to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline . Alternatively, it may be advantageous to administer an immunostimulant, such as an adjuvant, cytokine, protein, or other carrier with the vaccines or immunogenic compositions of the present invention. Therefore, this invention includes the use of such immunostimulants in conjunction with the compositions and methods of the present invention. An immunostimulant, as used herein, refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Said immunostimulants can be administered in the form of DNA or protein. Any of a variety of immunostimulants may be employed in conjunction with the vaccines and immunogenic compositions of the present inventions, including, but not limited to: GM-CSF, IFNα, tetanus toxoid, IL12, B7.1, LFA-3 and ICAM-1. Said immunostimulants are well-known in the art. Agents which assist in the cellular uptake of DNA, such as, but not limited to calcium ion, may also be used. These agents are generally referred to as transfection facilitating reagents and pharmaceutically acceptable carriers. Those of skill in the art will be able to determine the particular immunostimulant or pharmaceutically acceptable carrier as well as the appropriate time and mode of administration.

Any of a variety of procedures may be used to clone rhCEA. These methods include, but are not limited to, (1) a RACE PCR cloning technique (Frohman et al., *Proc. Natl. Acad. Sci. USA* 85: 8998-9002 (1988)). 5′ and/or 3′ RACE may be

performed to generate a full-length cDNA sequence. This strategy involves using gene-specific oligonucleotide primers for PCR amplification of rhCEA cDNA. These gene-specific primers are designed through identification of an expressed sequence tag (EST) nucleotide sequence which has been identified by searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the rhCEA cDNA following the construction of a rhCEA-containing cDNA library in an appropriate expression vector system; (3) screening an rhCEA-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from the amino acid sequence of the rhCEA protein; (4) screening an rhCEA-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the rhCEA protein. This partial cDNA is obtained by the specific PCR amplification of rhCEA DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other membrane proteins which are related to the rhCEA protein; (5) screening a rhCEA-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA or oligonucleotide with homology to a mammalian rhCEA protein. This strategy may also involve using gene-specific oligonucleotide primers for PCR amplification of rhCEA cDNA identified as an EST as described above; or (6) designing 5′ and 3′ gene specific oligonucleotides using SEQ ID NO: 1 as a template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding rhCEA.

It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types-or species types, may be useful for isolating a rhCEA-encoding DNA or a rhCEA homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells. The selection of cells or cell lines for use in preparing a cDNA library to isolate a cDNA encoding rhCEA may be done by first measuring cell-associated rhCEA activity using any known assay available for such a purpose.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Sambrook et al., *Molecular Cloning: A Laboratory*

Manual; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989. Complementary DNA libraries may also be obtained from numerous commercial sources, including but not limited to Clontech Laboratories, Inc. (Palo Alto, CA) and Stratagene (La Jolla, CA).

The DNA molecules, RNA molecules, and recombinant protein of the present invention may be used to screen and measure levels of rhCEA. The recombinant proteins, DNA molecules, and RNA molecules lend themselves to the formulation of kits suitable for the detection and typing of rhCEA. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant rhCEA or anti-rhCEA antibodies suitable for detecting rhCEA. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing methodologies and materials that might be used in connection with the present invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be effected therein by one skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

The following examples illustrate, but do not limit the invention.

EXAMPLE 1

Isolation of RNA from Rhesus Macaques

Molecular procedures were performed following standard procedures well known in the art (*See, e.g.*, Ausubel et. al. *Short Protocols in Molecular Biology*, F.M., -2nd. ed., John Wiley & Sons, (1992) and Sambrook et al., *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press (1989), which are hereby incorporated by reference).

To obtain RNA for the isolation of the rhesus CEA cDNA, colon samples from two different Rhesus monkeys (*Macaca Mulatta*) were used. Frozen tissues were obtained from The Biomedical Primate Research Center (BPRC, Rijswijk, the Netherlands). To extract total RNA from rhesus colon samples, tissues were mechanically pulverized and combined with the Ultraspec RNA reagent (Biotecx Laboratories; Houston, TX) according to the manufacturer's instructions. The integrity of the purified RNA was verified by formaldehyde-denaturing agarose gel. Samples were aliquoted and stored at -80°C.

EXAMPLE 2

Rhesus CEA cDNA Amplification

Nucleotide sequences from the 5' and 3' untranslated regions (UTR) of all known members of the human CEA family were aligned to identify highly conserved regions of the CEA DNA (see FIGURE 3). Based on the CEA gene family homologies identified, degenerate oligonucleotide primers were designed and PCR conditions were optimized to amplify the rhesus CEA cDNA by reverse transcriptase polymerase chain reaction (RT-PCR), described below. The primers used to amplify the entire cDNA were as follows: 5'-RhCEA EcoRI 5'- C C G A A T T C C G G A C A S A G C A G R C A G C A G R S A C C -3' (SEQ ID NO:3) and CEA-8 RhXhoI 5'- C C G C T C G A G C G G C T G C T A C A T C A G A G C A A C C C C A A C C -3' (SEQ ID NO:4). The amplification was performed with the SuperScript One-Step RT-PCR with Platinum Taq kit (Invitrogen; Carlsbad, CA). A 100 µl reaction volume was used which consisted of 1 µg of RNA, 200pmol of both primers, and 10% DMSO (final concentration).

To perform the reverse transcription step, total RNA samples isolated from each of the two rhesus monkeys were incubated at 45°C for 30 min, followed by a 2 minute incubation at 94°C. PCR amplification of the resulting templates consisted of 40 cycles of 94°C for 15s, 52°C for 30s and 68°C for 2 min and 20s.

Amplified PCR products of about 2100 bp, the expected size for a CEACAM-5 homolog, were independently obtained from both RNA samples and were purifed from agarose gel. Partial sequence analysis of both PCR products revealed high homology with human CEACAM-5.

Due to the high homology of internal repetitions, the entire gene sequence was obtained by purifying DNA fragments using the restriction sites indicated in FIGURE 5. The rhesus CEA nucleotide sequences obtained from each monkey are disclosed herein in FIGURE 1, as set forth in SEQ ID NO:1 (hereinafter rhCEACAM-5) and SEQ ID NO:5 (hereinafter rhCEACAM-5 #2). Analysis of the CEA nucleotide sequences revealed an open reading frame (ORF) of 2118 nucleotides, which encode a 705 amino acid polypeptide. Comparison of the rhCEA nucleotide sequences obtained from two rhesus monkeys indicated that there were two nucleotide differences (see FIGURES 1A and 1B), which code for two different proteins (see FIGURES 2A and 2B).

The rhesus CEACAM-5 nucleotide sequence (SEQ ID NO:1) was also compared to the published human CEACAM-5 sequence (SEQ ID NO:6), which revealed 88% homology at the nucleotide level (see FIGURE 6). A similar comparison of the rhesus (SEQ ID NO:2) and human (SEQ ID NO:7) CEA polypeptide sequences showed 78.9% identity at the amino acid level (see FIGURE 7). Interestingly, a three amino acid insertion is present in the carboxyl-terminus of rhesus CEA compared to human CEA, probably involving the signal for glycosylphosphatidylinositol (GPI) modification.

EXAMPLE 3

Generation and Screening of a Lambda Rhesus CEA-Specific Library.

Amplified rhCEA products obtained by RT-PCR (see EXAMPLE 2) were digested with *Eco*RI/*Xho*I and ligated into the Lambda ZAP-CMV XR vector (Stratagene; La Jolla, CA), according to manufacturer's directions. The ligation products were incubated with Gigapack III gold packaging extract and the resulting phages were used to infect XL-1 Blue MRF' cells. This CEA-specific primary library was then amplified, obtaining a titer of ~$1x10^6$ pfu/ml. Screening of ~$5x10^3$ plaques was performed by lifting onto nylon filters. Filters were hybridized with two different DNA probes covering the 5' and the 3' ends of the CEA molecule. Double positive plaques were excised in XL-1 Blue MRF' cells and the derived filamentous phages were amplified in XL-OLR cells. The phagemids were then grown and analyzed by restriction digestion. Sequence analysis and Genbank comparisons revealed the highest homology with human CEACAM-5.

EXAMPLE 4

Plasmid Constructs and Adenovirus Generation

RhCEA was excised with *PstI/XhoI* from pCMV-script EX phagemid vector and inserted in pBluescript II KS vector, obtaining pBS-RhCEA. The insert was entirely sequenced and then subcloned as *SmaI/XhoI* fragment in pVIJnsA vector, obtaining pVIJ-RhCEA. The shuttle plasmid pMRK-RhCEA for adenovirus generation was obtained by subcloning the same fragment in the polyMRK vector. A *PacI/StuI* fragment from pMRK-RhCEA containing the expression cassette for RhCEA and E1 flanking Ad5 regions was recombined to *ClaI* linearized pAd5 or pAd6 in BJ5183 *E. Coli* cells. The resulting plasmids were pAd5-RhCEA and pAd6-RhCEA. Both plasmids were cut with *Pac*I to release the adenovirus ITRs and transfected in PerC-6 cells. Viral amplification was carried out through serial passages. Ad5-RhCEA and Ad6-RhCEA were purified using a standard CsCl purification protocol and extensively dyalized against A105 buffer (5mM Tris pH 8.0, 1mM MgCl2, 75mM NaCl, 5% sucrose, 0.005% Tween20).

EXAMPLE 5

RhCEA Expression and Detection *in vitro*

Expression of RhCEA by the generated vectors was verified by western blot and FACS analysis. Plasmids were transfected in HeLa or PerC.6 cells with Lipofectamine 2000 (Life Technologies; Carlsbad, CA). Adenovirus infections were performed in serum-free medium for 30 min at 37°C, then fresh medium was added. After 48hr of incubation, whole cell lysates were analyzed by western blot using a rabbit polyclonal serum against human CEA (Fitzgerald, 1:1500 dilution). All of the selected rhesus CEA clones expressed a 180-200 KDa protein when transfected in HeLa cells (see FIGURE 4).

For FACS analysis, cells were detached with trypsin and resuspended in FACS buffer (PBS, 1% FCS). After incubation for 30 min with rabbit polyclonal anti-CEA antibody diluted 1:250, cells were washed and incubated for 30 min with an

anti-rabbit IgG-PE and finally analyzed with a FACScalibur (Becton Dickinson, San Jose, CA).

EXAMPLE 6

Peptides

In order to analyze the cell mediated immune response against rhesus CEA in immunized animals, 15mer peptides overlapping by 11 amino acids were designed to cover the entire protein. Liophylized rhesus CEA peptides were purchased by Bio-Synthesis, Inc. (Lewisville, TX) and resuspended in DMSO at 40mg/ml. Peptides were grouped into 4 pools: pool A (from RhCEA-1 to RhCEA-34, 34 peptides); pool B (from RhCEA-35 to RhCEA-79, 45 peptides); pool C (from RhCEA-80 to RhCEA-124, 48 peptides); and pool D (from RhCEA-125 to RhCEA-173, 53 peptides). Final concentrations were the following: pool A=1.176mg/ml; pool B=0.888mg/ml; pool C=0.851mg/ml; pool D=0.769mg/ml. Peptides and pools were stored at -80°C.

EXAMPLE 7

Generation of CEA-specific cellular immune responses in mice by immunization with rhCEA

CEA.Tg mice are transgenic mice that express human CEA as a self-antigen with a tissue distribution similar to that of humans. As largely demonstrated in the scientific literature, these mice are unresponsive to CEA, as shown by the lack of detectable CEA-specific serum antibodies and the inability to prime an *in vitro* splenic T-cell response to CEA. Many reports have shown that DNA immunization with xenogeneic genes encoding homologous antigens protects mice against tumor challenge with syngeneic melanoma cells. To demonstrate the capability of xenogeneic DNA vaccination to elicit an immune response against a self-antigen in this model, we immunized CEA.Tg mice with vectors encoding rhesus CEA (xeno).

C57BL/6 mice (H-2^b) were purchased from Charles River (Lecco, Italy). CEA.tg mice (H-2^b) were provided by HL Kaufman (Albert Einstein College of Medicine, New York) and kept in standard conditions.

For electro gene transfer (EGT), mice quadriceps were either surgically exposed or directly injected with 50 μg pVIJ-RhCEA and electrically stimulated as previously described (Rizzuto at al. *Proc. Natl. Acad. Sci. U.S.A.* 96(11): 6417-22 (1999)). For adenovirus injection, $1x10^{10}$ vp of Ad5-RhCEA were injected in mice quadriceps.

Mice were injected in the quadriceps muscle with 50μg pVIJ-RhCEA and electrostimulated immediately after injection once a week for 4 weeks. C57BL/6 mice were used as controls. Antibodies against rhesus CEA were detected in sera from these mice by western blot, demonstrating a humoral immune response. A mouse monoclonal Ab against hCEA was used as positive control, while pre-immune sera and mock-infected cell extracts were used as negative controls (data not shown). Importantly, cross-reactive antibodies against human CEA protein could be measured only in rhesus CEA immunized groups (FIGURE 8) with an average titer of 1:110. These data indicate that, in the transgenic mouse model, it is possible to break tolerance with xenogeneic DNA vaccination (measured as anti-CEA autoantibodies).

EXAMPLE 8

Antibody Detection and Titration

Sera for antibody titration were obtained by retro-orbital bleeding. For western blot detection, extracts from HeLa cells transduced with Ad5-rhCEA were run on SDS-page gels and transferred onto nitrocellulose filters. Sera were pooled and diluted 1:50 for O/N incubation at 4°C. An anti-mouse IgG-AP conj. (Sigma, 1:2500) was used for the detection. For titration, Elisa plates (Nunc maxisorp) were coated with 100ng/well CEA (highly pure CEA; Fitzgerald Industries International Inc., Concord MA), diluted in coating buffer (50mM $NaHCO_3$ pH 9.4) and incubated O/N at 4°C. Plates were then blocked with PBS containing 5% BSA for 1 hr at 37°C. Mouse sera were diluted in PBS 5% BSA (dilution 1/50 to evaluate seroconversion rate; dilutions from 1:10 to 1:31,250 to evaluate titre value). Pre-immune sera were used as background. Diluted sera were incubated O/N at 4°C. Washes were carried out with PBS, 1%BSA, 0.05% tween 20. Detecting antibody (goat anti-mouse IgG

Peroxidase, Sigma, St. Louis, MO) was diluted 1/2000 in PBS, 5%BSA.) and incubated for 2-3 hr at room temp. on a shaker. After washing, plates were developed with 100µl/well of TMB substrate (Pierce Biotechnology, Inc., Rockford, IL). Reactions were stopped with 25µl/well of 1M H_2SO_4 solution and plates were read at 450 nm/620 nm. Anti-CEA serum titers were calculated as the limiting dilution of serum producing an absorbance at least 3-fold greater than the absorbance of autologous pre-immune serum at the same dilution.

EXAMPLE 9

IFN-γ ELISPOT Assay

96-well MAIP plates (Millipore, Bedford, MA) were coated with purified rat anti-mouse IFN-γ (IgG1, clone R4-6A2, Pharmingen, San Diego, CA) at 2.5µg/ml in sterile PBS, aliquoted at 100µl per well. After washing with sterile PBS, plates were blocked with 200µl per well of R10 medium at 37 °C for at least 2 hours.

For splenocyte preparation, the spleen was removed from a sacrificed mouse in a sterile manner and disrupted by scratching through a grid. Osmotic lysis of red blood cells was obtained by adding 1 ml of 0.1X PBS to the cell pellet and vortexing for no more than 15 sec. 1 ml of 2X PBS was then added and the volume was brought up to 4ml with PBS 1X. After spinning at 1200 rpm for 10 minutes at room temp., the cell pellet was resuspended in 1 ml of R10 medium and viable cells were counted. Splenocytes were plated at $5x10^5$ and $2x10^5$/well with 1µg/ml each peptide in R10 and incubated for 20h in a CO_2 incubator at 37°C. Concanavalin A (ConA) at 5µg/ml was used as a positive internal control for each mouse. After washing with PBS, 0.05% Tween 20, plates were incubated O/N at 4°C with 50µl/well of biotin-conjugated rat anti-mouse IFN-γ (Rat IgG1, clone XMG 1.2, Pharmingen, San Jose, CA) diluted 1:250 in assay buffer (PBS-5%FBS - 0.005% Tween-20).

The next day, plates were washed and incubated for 2h at room temp. with Streptavidin-AP conjugate (Pharmingen) diluted 1:2500 in assay buffer. After extensive washing, plates were developed by addition of 50µl/well NBT/B-CIP (Pierce Biotechnology) until development of spots was observed under the microscope. The reaction was stopped by washing plates thoroughly with distilled

water. Plates were allowed to air-dry completely, and spots were counted using an automated ELISPOT reader.

For cell mediated immune response, CEA.Tg mice were vaccinated either with hCEA expressing vectors or with rhCEA expressing vectors. Two groups were analyzed: the first group was analyzed by ELISPOT assay 21 days after last DNA injection, while the second group was boosted with $1x10^{10}$ vp of either Ad5-hCEA or Ad5-RhCEA and analyzed two weeks later. Results demonstrated that after four DNA injections, no significant cellular immune-response against hCEA was observed as measured by ELISPOT (not shown). On the other hand, mice that were boosted with Ad5 demonstrated a considerably increased response, consistent with breaking the immune-tolerance to CEA. This observation suggests that a useful vaccination protocol for the CEA self antigen would be the repeated administration of DNA by EGT, followed by an adenovirus boost (mixed modality). Importantly, immunization with rhesus CEA provided cross-reaction with human CEA peptides and vice-versa both in wild type and transgenic mice (data not shown). In particular, the immune response against human CEA was much better in transgenic mice using rhCEA as the immunogen (see FIGURE 9). These results show that a good response against CEA in transgenic mice could be obtained using the rhesus (xeno) gene. Response against rhesus CEA peptides is shown in FIGURE 10.

EXAMPLE 10

Immunization of Rhesus Macaques with rhCEA

In order to assess the efficiency of immunization of rhesus macaques (*macaca mulatta*) with the rhesus homologue of the human tumour antigen CEA, which is expressed in colorectal carcinomas, immunization studies were performed at the Biomedical Primate Research Centre (BPRC, Rijswijk, The Netherlands). Such immunization studies were designed to evaluate both B and T cell responses to immunization with the rhesus CEA antigen.

In this study (CV-1), 1 group of monkeys (consisting of 2 males and 2 females) was immunized with a plasmid DNA vector and adenovirus vector expressing rhesus CEACAM-5. For priming, animals were vaccinated intramuscularly with plasmid DNA expressing rhCEA at weeks 0, 4, 8, 12, and 16 by injection of DNA followed by electrical stimulation. The DNA injection consisted of

a 1 ml solution (split over 2 sites with 0.5 ml/site) containing 5 mg plasmid DNA for animals weighing 2-5 kilos. Animals were injected under anesthesia (mixture of ketamine/xylazine).

For electrostimulation, 2 trains of 100 square bipolar pulses (1 sec each), were delivered every other second for a total treatment time of 3 sec. The pulse length was 2 msec/phase with a pulse frequency and amplitude of 100 Hz and 100 mA (constant current mode), respectively.

To measure the immune response to CEA using the above immunization protocol, blood samples were collected every four weeks. The cell mediated response was measured by IFNγ Elispot assay and the humoral response was measured by ELISA assay. Because no significant immune response was obtained at week 16, two further injections (week 24 and 28) were carried out using Ad5 expressing rhCEA. Upon Ad5 injection, a measurable immune response against rhCEA was detected for two monkeys (RI137 and CO12) covering peptide pool C and pool B + C, respectively. The cell mediated immune response began to decline in both monkeys at week 35.

The humoral immune response was followed over time upon DNA injection. Three monkeys (CO12, RI311 and RI002) showed a good anti-CEA antibody titer, ranging from 1:143 to 1:2099 and reaching a peak between weeks 12 and 16 after the first injection.

These data show that genetic vectors encoding rhCEA were able to break the immune tolerance to this tumor antigen in primates. Both cell mediated (50% of treated monkeys) and humoral (75% of treated monkeys) immunity were involved in the immune response.

EXAMPLE 11

Immunization of Rhesus Macaques with Rhesus Homologs of Human Tumor-Associated Antigens

A second series of immunization studies was performed in order to assess the efficiency of immunization of Rhesus macaques (*Macaca mulatta*) with rhesus homologues of the human tumor antigens HER2/neu, Ep-CAM and CEA, which are all expressed in colorectal carcinomas. Protocols were designed to evaluate both B and T cell responses to these tumor antigens in combination.

In this study, a second group of 4 rhesus monkeys (2 males and 2 females) were immunized with a mixture of three plasmid DNA vectors expressing the rhesus homologues of human tumor antigens Ep-CAM (pV1J-rhEpCAM), CEA pV1J-rhCEA), and HER2/neu (pV1J-rhHER2).

Animals were primed by intramuscular injection of plasmid DNA at weeks 0, 4, 8, 12, and 16, followed by electrostimulation. The DNA injection consisted of a 1 ml solution (split over 2 sites with 0.5 ml/site) containing 6 mg plasmid DNA for animals weighing 2-5 kilos. Animals were injected under anesthesia (mixture of ketamine/xylazine).

For electrostimulation, 2 trains of 100 square bipolar pulses (1 sec each), were delivered every other second for a total treatment time of 3 sec. The pulse length was 2 msec/phase with a pulse frequency and amplitude of 100 Hz and 100 mA (constant current mode), respectively.

The same group of animals was boosted by injection of a mixture of three Ad5-expressing rhesus CEA (Ad5-rhCEA), rhesus HER2/neu (Ad5-rhHER2), and rhesus EpCAM (Ad5-rhEpCAM). A total amount of 3x10exp11 viral particles (vp), were injected i.m. at weeks 23 and 27 (1x10exp11 vp for each of the three viruses).

To measure the immune response to the three tumor antigens using the above immunization protocol, blood samples were collected every four weeks. The cell mediated immune response was measured by IFN-γ+ ELISPOT assay, whereas the humoral response was measured by ELISA.

Monkeys RI449 and RI519 showed a detectable HER2-specific cell-mediated response, as measured by IFN-γ ELISPOT analysis. A similar analysis did not detect any significant response against rhCEA and rhEpCAM.

In a third study, 4 rhesus monkeys were immunized with a mixture of Ad5-rhHER2, Ad5-rhCEA and Ad5-rhEpCAM by i.m. injection of Ad5 derivatives at weeks 0, 2 and 4. A 1 ml solution (split over 2 sites with 0.5 ml/site) containing 3x10exp11 vp (10exp11 for each of the three Ad5 virus) was administered to animals weighing 2-5 kilos, under anesthesia (mixture of ketamine/xylazine).

The cell mediated response was measured by IFNγ ELISPOT assay. For Her2/Neu, three out of four monkeys showed a detectable response. No significant cell mediated responses were measured for rhCEA and rhEpCAM.

In summary, the immunization protocol discussed above was effective in inducing a specific immune response against rhHER2/neu in rhesus monkeys. It is

unclear why co-immunization with vectors carrying three different tumour antigens was not effective in inducing an innume response against rhCEA, as compared to study 1, which used only rhCEA as immunogen. Though not wishing to be bound by theory, it is possible that the expression of rhHER2/Neu and the presence of immunodominant epitopes limited the generation and the expansion of subdominant rhCEA specific T-cells.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule, comprising a sequence of nucleotides that encodes a rhesus monkey carcinoembryonic antigen (rhCEA) protein as set forth in SEQ ID NO:2.

2. The isolated nucleic acid molecule of claim 1 wherein the nucleic acid is DNA.

3. The isolated nucleic acid molecule of claim 1 wherein the nucleic acid is mRNA.

4. The isolated nucleic acid molecule of claim 1 wherein the nucleic acid is cDNA.

5. The isolated nucleic acid molecule of claim 1 wherein the sequence of nucleotides comprises the sequence of nucleotides set forth in SEQ ID NO:1.

6. A vector comprising the nucleic acid molecule of claim 1.

7. A host cell comprising the vector of claim 6.

8. A process for expressing a rhesus carcinoembryonic antigen (CEA) protein in a recombinant host cell, comprising:

(a) introducing a vector comprising the nucleic acid of claim 1 into a suitable host cell; and,

(b) culturing the host cell under conditions which allow expression of said rhesus CEA protein.

9. A process for expressing a rhesus carcinoembryonic antigen (CEA) protein in a recombinant host cell, comprising:

(a) introducing a vector comprising the nucleic acid of claim 5 into a suitable host cell; and,

(b) culturing the host cell under conditions which allow expression of said rhesus CEA protein.

10. An isolated and purified rhesus CEA polypeptide comprising a sequence of amino acids as set forth in SEQ ID NO:2.

11. An isolated nucleic acid molecule, comprising a sequence of nucleotides that encodes a rhesus monkey carcinoembryonic antigen (rhCEA) protein as set forth in SEQ ID NO:18.

12. The isolated nucleic acid molecule of claim 11 wherein the nucleic acid is DNA.

13. The isolated nucleic acid molecule of claim 11 wherein the nucleic acid is mRNA.

14. The isolated nucleic acid molecule of claim 11 wherein the nucleic acid is cDNA.

15. The isolated nucleic acid molecule of claim 11 wherein the sequence of nucleotides comprises the sequence of nucleotides set forth in SEQ ID NO:5.

16. A vector comprising the nucleic acid molecule of claim 11.

17. A host cell comprising the vector of claim 16.

18. A process for expressing a rhesus carcinoembryonic antigen (CEA) protein in a recombinant host cell, comprising:

(a) introducing a vector comprising the nucleic acid of claim 11 into a suitable host cell; and,

(b) culturing the host cell under conditions which allow expression of said rhesus CEA protein.

19. An isolated and purified rhesus CEA polypeptide comprising a sequence of amino acids as set forth in SEQ ID NO:18.

20. A method of preventing or treating cancer comprising administering to a mammal a vaccine vector comprising an isolated nucleic acid molecule, the isolated nucleic acid molecule comprising a sequence of nucleotides that encodes a rhesus monkey carcinoembryonic antigen (rhCEA) protein as set forth in SEQ ID NO:2 or SEQ ID NO:18.

21. A method according to claim 20 wherein the mammal is human.

22. A method according to claim 20 wherein the vector is an adenovirus vector or a plasmid vector.

23. A method according to claim 20 wherein the vector is an adenoviral vector comprising an adenoviral genome with a deletion in the adenovirus E1 region, and an insert in the adenovirus E1 region, wherein the insert comprises an expression cassette comprising:

(a) a polynucleotide encoding a rhesus monkey CEA protein; and

(b) a promoter operably linked to the polynucleotide.

24. A method according to claim 20 wherein the vector is a plasmid vaccine vector, which comprises a plasmid portion and an expressible cassette comprising

(a) a polynucleotide encoding a rhesus monkey CEA protein; and

(b) a promoter operably linked to the polynucleotide.

25. An adenovirus vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising:

(a) a polynucleotide encoding a rhesus monkey CEA protein; and

(b) a promoter operably linked to the polynucleotide.

26. An adenovirus vector according to claim 25 which is an Ad 5 vector.

27. An adenovirus vector according to claim 25 which is an Ad 6 vector.

28. A vaccine plasmid comprising a plasmid portion and an expression cassette portion, the expression cassette portion comprising:

(a) a polynucleotide encoding a rhesus monkey CEA protein; and

(b) a promoter operably linked to the polynucleotide.

29. A method of protecting a mammal from cancer comprising:

(a) introducing into the mammal a first vector comprising:

(i) a polynucleotide encoding a rhesus monkey CEA protein; and

(ii) a promoter operably linked to the polynucleotide;

(b) allowing a predetermined amount of time to pass; and

(c) introducing into the mammal a second vector comprising:

(i) a polynucleotide encoding a rhesus monkey CEA protein; and

(ii) a promoter operably linked to the polynucleotide.

30. A method according to claim 29 wherein the first vector is a plasmid and the second vector is an adenovirus vector.

31. A method according to claim 29 wherein the first vector is an adenovirus vector and the second vector is a plasmid.

32. A method of treating a mammal suffering from a colorectal carcinoma comprising:

(a) introducing into the mammal a first vector comprising:

(i) a polynucleotide encoding a rhesus monkey CEA protein; and

(ii) a promoter operably linked to the polynucleotide;

(b) allowing a predetermined amount of time to pass; and

(c) introducing into the mammal a second vector comprising:

(i) a polynucleotide encoding a rhesus monkey CEA protein; and

(ii) a promoter operably linked to the polynucleotide.

33. A method according to claim 32 wherein the first vector is a plasmid and the second vector is an adenovirus vector.

34. A method according to claim 32 wherein the first vector is an adenovirus vector and the second vector is a plasmid.

Nucleotide Sequence of First Rhesus CEA

```
   1 ATGGGGTCTC CCTCAGCCCC TCTTCACAGA TGGTGCATCC CCTGGCAGAC
  51 GCTCCTGCTC ACAGCCTCAC TTCTAACCTT CTGGAACCCG CCCACCACTG
 101 CCCAGCTCAC TATTGAATCC AGGCCGTTCA ATGTTGCAGA GGGGAAGGAG
 151 GTTCTTCTAC TTGCCCACAA TGTGTCCCAG AATCTTTTTG GCTACATTTG
 201 GTACAAGGGA GAAAGAGTGG ATGCCAGCCG TCGAATTGGA TCATGTGTAA
 251 TAAGAACTCA ACAAATTACC CCAGGGCCCG CACACAGCGG TCGAGAGACA
 301 ATAGACTTCA ATGCATCCCT GCTGATCCAC AATGTCACCC AGAGTGACAC
 351 AGGATCCTAC ACCATACAAG TCATAAAGGA AGATCTTGTG AATGAAGAAG
 401 CAACTGGCCA GTTCCGGGTA TACCCGGAGC TGCCCAAGCC CTACATCTCC
 451 AGCAACAACT CCAACCCCGT GGAGGACAAG GATGCTGTGG CCTTAACCTG
 501 TGAACCTGAG ACTCAGGACA CAACCTACCT GTGGTGGGTA AACAATCAGA
 551 GCCTCCCGGT CAGTCCCAGG CTGGAGCTGT CCAGTGACAA CAGGACCCTC
 601 ACTGTATTCA ATATTCCAAG AAATGACACA ACATCCTACA AATGTGAAAC
 651 CCAGAACCCA GTGAGTGTCA GACGCAGCGA CCCAGTCACC CTGAACGTCC
 701 TCTATGGCCC GGATGCGCCC ACCATTTCCC CTCTAAACAC ACCTTACAGA
 751 GCAGGGGAAA ATCTGAACCT CACCTGCCAC GCAGCCTCTA ACCCAACTGC
 801 ACAGTACTTT TGGTTTGTCA ATGGGACGTT CCAGCAATCC ACACAAGAGC
 851 TCTTTATACC CAACATCACC GTGAATAATA GCGGATCCTA TATGTGCCAA
 901 GCCCATAACT CAGCCACTGG CCTCAATAGG ACCACAGTCA CGGCGATCAC
 951 AGTCTACGCG GAGCTGCCCA AGCCCTACAT CACCAGCAAC AACTCCAACC
1001 CCATAGAGGA CAAGGATGCT GTGACCTTAA CCTGTGAACC TGAGACTCAG
1051 GACACAACCT ACCTGTGGTG GGTAAACAAT CAGAGCCTCT CGGTCAGTTC
1101 CAGGCTGGAG CTGTCCAATG ACAACAGGAC CCTCACTGTA TTCAATATTC
1151 CAAGAAACGA CACAACGTTC TACGAATGTG AGACCCAGAA CCCAGTGAGT
1201 GTCAGACGCA GCGACCCAGT CACCCTGAAT GTCCTCTATG GCCCGGATGC
1251 GCCCACCATT TCCCCTCTAA ACACACCTTA CAGAGCAGGG GAAAATCTGA
1301 ACCTCTCCTG CCACGCAGCC TCTAACCCAG CTGCACAGTA CTCTTGGTTT
1351 GTCAATGGGA CGTTCCAGCA ATCCACACAA GAGCTCTTTA TACCCAACAT
1401 CACCGTGAAT AATAGCGGAT CCTATATGTG CCAAGCCCAT AACTCAGCCA
1451 CTGGCCTCAA TAGGACCACA GTCACGGCGA TCACAGTCTA TGTGGAGCTG
1501 CCCAAGCCCT ACATCTCCAG CAACAACTCC AACCCCATAG AGGACAAGGA
1551 TGCTGTGACC TTAACCTGTG AACCTGTGGC TGAGAACACA ACCTACCTGT
1601 GGTGGGTAAA CAATCAGAGC CTCTCGGTCA GTCCCAGGCT GCAGCTCTCC
1651 AATGGCAACA GGATCCTCAC TCTACTCAGT GTCACACGGA ATGACACAGG
1701 ACCCTATGAA TGTGGAATCC AGAACTCAGA GAGTGCAAAA CGCAGTGACC
1751 CAGTCACCCT GAATGTCACC TATGGCCCAG ACACCCCCAT CATATCCCCC
1801 CCAGACTTGT CTTACCGTTC GGGAGCAAAC CTCAACCTCT CCTGCCACTC
1851 GGACTCTAAC CCATCCCCGC AGTATTCTTG GCTTATCAAT GGGACACTGC
1901 GGCAACACAC ACAAGTTCTC TTTATCTCCA AAATCACATC AAACAATAGC
1951 GGGGCCTATG CCTGTTTTGT CTCTAACTTG GCTACCGGTC GCAATAACTC
2001 CATAGTCAAG AACATCTCAG TCTCCTCTGG CGATTCAGCA CCTGGAAGTT
2051 CTGGTCTCTC AGCTAGGGCT ACTGTCGGCA TCATAATTGG AATGCTGGTT
2101 GGGGTTGCTC TGATGTAG (SEQ ID NO:1)
```

FIG.1A

Nucleotide Sequence of Second Rhesus CEA

```
   1  ATGGGGTCTC CCTCAGCCCC TCTTCACAGA TGGTGCATCC CCTGGCAGAC
  51  GCTCCTGCTC ACAGCCTCAC TTCTAACCTT CTGGAACCCG CCCACCACTG
 101  CCCAGCTCAC TATTGAATCC AGGCCGTTCA ATGTTGCAGA GGGGAAGGAG
 151  GTTCTTCTAC TTGCCCACAA TGTGTCCCAG AATCTTTTTG GCTACATTTG
 201  GTACAAGGGA GAAAGAGTGG ATGCCAGCCG TCGAATTGGA TCATGTGTAA
 251  TAAGAACTCA ACAAATTACC CCAGGGCCCG CACACAGCGG TCGAGAGACA
 301  ATAGACTTCA ATGCATCCCT GCTGATCCAC AATGTCACCC AGAGTGACAC
 351  AGGATCCTAC ACCATACAAG TCATAAAGGA AGATCTTGTG AATGAAGAAG
 401  CAACTGGCCA GTTCCGGGTA TACCCGGAGC TGCCCAAGCC CTACATCTCC
 451  AGCAACAACT CCAACCCCGT GGAGGACAAG GATGCTGTGG CCTTAACCTG
 501  TGAACCTGAG ACTCAGGACA CAACCTACCT GTGGTGGGTA AACAATCAGA
 551  GCCTCCCGGT CAGTCCCAGG CTGGAGCTGT CCAGTGACAA CAGGACCCTC
 601  ACTGTATTCA ATATTCCAAG AAATGACACA ACATCCTACA AATGTGAAAC
 651  CCAGAACCCA GTGAGTGTCA GACGCAGCGA CCCAGTCACC CTGAACGTCC
 701  TCTATGGCCC GGATGCGCCC ACCATTTCCC CTCTAAACAC ACCTTACAGA
 751  GCAGGGGAAA ATCTGAACCT CACCTGCCAC GCAGCCTCTA ACCCAACTGC
 801  ACAGTACTTT TGGTTTGTCA ATGGGACGTT CCAGCAATCC ACACAAGAGC
 851  TCTTTATACC CAACATCACC GTGAATAATA GCGGATCCTA TATGTGCCAA
 901  GCCCATAACT CAGCCACTGG CCTCAATAGG ACCACAGTCA CGGCGATCAC
 951  AGTCTACGCG GAGCTGCCCA AGCCCTACAT CACCAGCAAC AACTCCAACC
1001  CCATAGAGGA CAAGGATGCT GTGACCTTAA CCTGTGAACC TGAGACTCAG
1051  GACACAACCT ACCTGTGGTG GGTAAACAAT CAGAGCCTCT CGGTCAGTTC
1101  CAGGCTGGAG CTGTCCAATG ACAACAGGAC CCTCACTGTA TTCAATATTC
1151  CAAGAAACGA CACAACGTTC TACGAATGTG AGACCCAGAA CCCAGTGAGT
1201  GTCAGACGCA GCGACCCAGT CACCCTGAAT GTCCTCTATG GCCCGGATGC
1251  GCCCACCATT TCCCCTCTAA ACACACCTTA CAGAGCAGGG GAAAATCTGA
1301  ACCTCTCCTG CCACGCAGCC TCTAACCCAG CTGCACAGTA CTTTTGGTTT
1351  GTCAATGGGA CGTTCCAGCA ATCCACACAA GAGCTCTTTA TACCCAACAT
1401  CACCGTGAAT AATAGCGGAT CCTATATGTG CCAAGCCCAT AACTCAGCCA
1451  CTGGCCTCAA TAGGACCACA GTCACGGCGA TCACAGTCTA TGTGGAGCTG
1501  CCCAAGCCCT ACATCTCCAG CAACAACTCC AACCCCATAG AGGACAAGGA
1551  TGCTGTGACC TTAACCTGTG AACCTGTGGC TGAGAACACA ACCTACCTGT
1601  GGTGGGTAAA CAATCAGAGC CTCTCGGTCA GTCCCAGGCT GCAGCTCTCC
1651  AATGGCAACA GGATCCTCAC TCTACTCAGT GTCACACGGA ATGACACAGG
1701  ACCCTATGAA TGTGGAATCC AGAACTCAGA GAGTGCAAAA CGCAGTGACC
1751  CAGTCACCCT GAATGTCACC TATGGCCCAG ACACCCCCAT CATATCCCCC
1801  CCAGACTTGT CTTACCGTTC GGGAGCAAAC CTCAACCTCT CCTGCCACTC
1851  GGACTCTAAC CCATCCCCGC AGTATTCTTG GCTTATCAAT GGGACACTGC
1901  GGCAACACAC ACAAGTTCTC TTTATCTCCA AAATCACATC AAACAATAAC
1951  GGGGCCTATG CCTGTTTTGT CTCTAACTTG GCTACCGGTC GCAATAACTC
2001  CATAGTCAAG AACATCTCAG TCTCCTCTGG CGATTCAGCA CCTGGAAGTT
2051  CTGGTCTCTC AGCTAGGGCT ACTGTCGGCA TCATAATTGG AATGCTGGTT
2101  GGGGTTGCTC TGATGTAG (SEQ ID NO:5)
```

FIG.1B

Predicted Amino Acid Sequence of First Rhesus CEA Protein

```
  1  MGSPSAPLHR WCIPWQTLLL TASLLTFWNP PTTAQLTIES RPFNVAEGKE
 51  VLLLAHNVSQ NLFGYIWYKG ERVDASRRIG SCVIRTQQIT PGPAHSGRET
101  IDFNASLLIH NVTQSDTGSY TIQVIKEDLV NEEATGQFRV YPELPKPYIS
151  SNNSNPVEDK DAVALTCEPE TQDTTYLWWV NNQSLPVSPR LELSSDNRTL
201  TVFNIPRNDT TSYKCETQNP VSVRRSDPVT LNVLYGPDAP TISPLNTPYR
251  AGENLNLTCH AASNPTAQYF WFVNGTFQQS TQELFIPNIT VNNSGSYMCQ
301  AHNSATGLNR TTVTAITVYA ELPKPYITSN NSNPIEDKDA VTLTCEPETQ
351  DTTYLWWVNN QSLSVSSRLE LSNDNRTLTV FNIPRNDTTF YECETQNPVS
401  VRRSDPVTLN VLYGPDAPTI SPLNTPYRAG ENLNLSCHAA SNPAAQYSWF
451  VNGTFQQSTQ ELFIPNITVN NSGSYMCQAH NSATGLNRTT VTAITVYVEL
501  PKPYISSNNS NPIEDKDAVT LTCEPVAENT TYLWWVNNQS LSVSPRLQLS
551  NGNRILTLLS VTRNDTGPYE CGIQNSESAK RSDPVTLNVT YGPDTPIISP
601  PDLSYRSGAN LNLSCHSDSN PSPQYSWLIN GTLRQHTQVL FISKITSNNS
651  GAYACFVSNL ATGRNNSIVK NISVSSGDSA PGSSGLSARA TVGIIIGMLV
701  GVALM (SEQ ID NO:2.)
```

FIG.2A

Predicted Amino Acid Sequence of Second Rhesus CEA Protein

```
  1  MGSPSAPLHR WCIPWQTLLL TASLLTFWNP PTTAQLTIES RPFNVAEGKE
 51  VLLLAHNVSQ NLFGYIWYKG ERVDASRRIG SCVIRTQQIT PGPAHSGRET
101  IDFNASLLIH NVTQSDTGSY TIQVIKEDLV NEEATGQFRV YPELPKPYIS
151  SNNSNPVEDK DAVALTCEPE TQDTTYLWWV NNQSLPVSPR LELSSDNRTL
201  TVFNIPRNDT TSYKCETQNP VSVRRSDPVT LNVLYGPDAP TISPLNTPYR
251  AGENLNLTCH AASNPTAQYF WFVNGTFQQS TQELFIPNIT VNNSGSYMCQ
301  AHNSATGLNR TTVTAITVYA ELPKPYITSN NSNPIEDKDA VTLTCEPETQ
351  DTTYLWWVNN QSLSVSSRLE LSNDNRTLTV FNIPRNDTTF YECETQNPVS
401  VRRSDPVTLN VLYGPDAPTI SPLNTPYRAG ENLNLSCHAA SNPAAQYFWF
451  VNGTFQQSTQ ELFIPNITVN NSGSYMCQAH NSATGLNRTT VTAITVYVEL
501  PKPYISSNNS NPIEDKDAVT LTCEPVAENT TYLWWVNNQS LSVSPRLQLS
551  NGNRILTLLS VTRNDTGPYE CGIQNSESAK RSDPVTLNVT YGPDTPIISP
601  PDLSYRSGAN LNLSCHSDSN PSPQYSWLIN GTLRQHTQVL FISKITSNNN
651  GAYACFVSNL ATGRNNSIVK NISVSSGDSA PGSSGLSARA TVGIIIGMLV
701  GVALM (SEQ ID NO:8)
```

FIG.2B

Alignment of 5' Untranslated Region of Human CEACAM Family Members

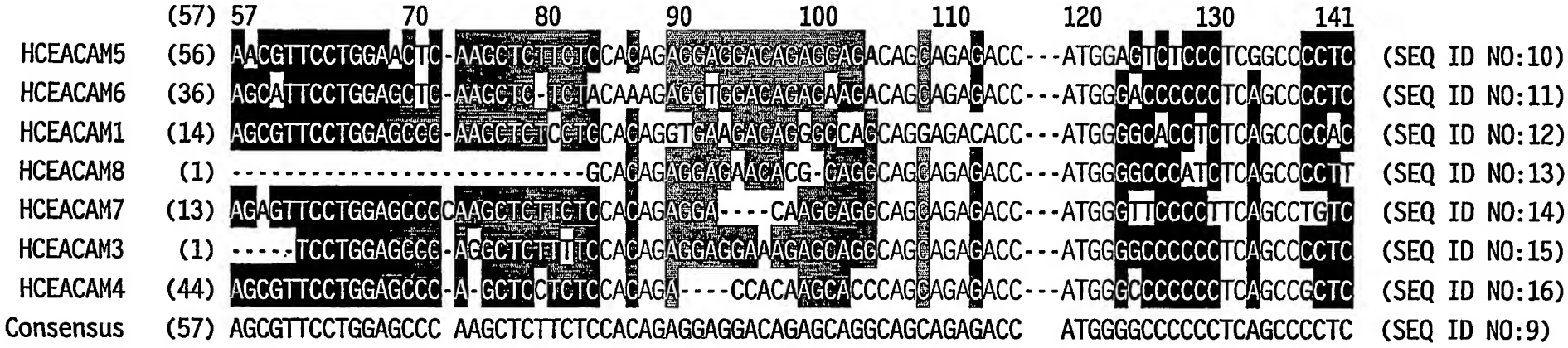

FIG.3

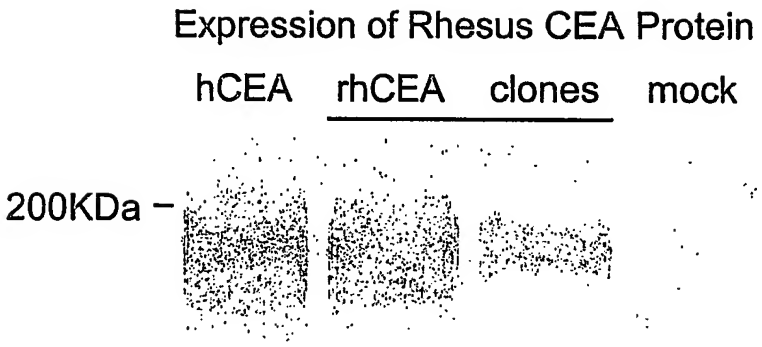
Expression of Rhesus CEA Protein
hCEA
rhCEA clones
mock
200KDa –

FIG.4

SCHEMATIC REPRESENTATION OF RHESUS CEA CODING SEQUENCE.

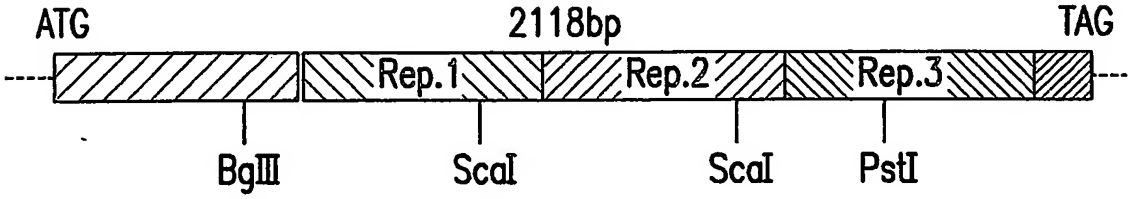

FIG.5

```
                (1) 1         10        20        30        40        50        60        70        80        90       100
Human CEACAM-5  (1) ATGGAGTCTCCCTCGGCCCCTCCCCACAGATGGTGCATCCCCTGGCAGAGGCTCCTGCTCACAGCCTCACTTCTAACCTTCTGGAACCCGCCCACCACTG
Rhesus CEACAM-5 (1) ATGGGGTCTCCCTCAGCCCCTCTTCACAGATGGTGCATCCCCTGGCAGACGCTCCTGCTCACAGCCTCACTTCTAACCTTCTGGAACCCGCCCACCACTG

               (101) 101       110       120       130       140       150       160       170       180       190       200
Human CEACAM-5 (101) CCAAGCTCACTATTGAATCCACGCCGTTCAATGTCGCAGAGGGGAAGGAGGTGCTTCTACTTGTCCACAATCTGCCCCAGCATCTTTTTGGCTACAGCTG
Rhesus CEACAM-5 (101) CCCAGCTCACTATTGAATCCAGGCCGTTCAATGTTGCAGAGGGGAAGGAGGTTCTTCTACTTGCCCACAATGTGTCCCAGAATCTTTTTGGCTACATTTG

               (201) 201       210       220       230       240       250       260       270       280       290       300
Human CEACAM-5 (201) GTACAAAGGTGAAAGAGTGGATGGCAACCGTCAAATTATAGGATATGTAATAGGAACTCAACAAGCTACCCCAGGGCCCGCATACAGTGGTCGAGAGATA
Rhesus CEACAM-5 (201) GTACAAGGGAGAAAGAGTGGATGCCAGCCGTCGAATTGGATCATGTGTAATAAGAACTCAACAAATTACCCCAGGGCCCGCACACAGCGGTCGAGAGACA

               (301) 301       310       320       330       340       350       360       370       380       390       400
Human CEACAM-5 (301) ATATACCCCAATGCATCCCTGCTGATCCAGAACATCATCCAGAATGACACAGGATTCTACACCCTACACGTCATAAAGTCAGATCTTGTGAATGAAGAAG
Rhesus CEACAM-5 (301) ATAGACTTCAATGCATCCCTGCTGATCCACAATGTCACCCAGAGTGACACAGGATCCTACACCATACAAGTCATAAAGGAAGATCTTGTGAATGAAGAAG

               (401) 401       410       420       430       440       450       460       470       480       490       500
Human CEACAM-5 (401) CAACTGGCCAGTTCCGGGTATACCCGGAGCTGCCCAAGCCCTCCATCTCCAGCAACAACTCCAAACCCGTGGAGGACAAGGATGCTGTGGCCTTCACCTG
Rhesus CEACAM-5 (401) CAACTGGCCAGTTCCGGGTATACCCGGAGCTGCCCAAGCCCTACATCTCCAGCAACAACTCCAACCCCGTGGAGGACAAGGATGCTGTGGCCTTAACCTG

               (501) 501       510       520       530       540       550       560       570       580       590       600
Human CEACAM-5 (501) TGAACCTGAGACTCAGGACGCAACCTACCTGTGGTGGGTAAACAATCAGAGCCTCCCGGTCAGTCCCAGGCTGCAGCTGTCCAATGGCAACAGGACCCTC
Rhesus CEACAM-5 (501) TGAACCTGAGACTCAGGACACAACCTACCTGTGGTGGGTAAACAATCAGAGCCTCCCGGTCAGTCCCAGGCTGGAGCTGTCCAGTGACAACAGGACCCTC

               (601) 601       610       620       630       640       650       660       670       680       690       700
Human CEACAM-5 (601) ACTCTATTCAATGTCACAAGAAATGACACAGCAAGCTACAAATGTGAAACCCAGAACCCAGTGAGTGCCAGGCGCAGTGATTCAGTCATCCTGAATGTCC
Rhesus CEACAM-5 (601) ACTGTATTCAATATTCCAAGAAATGACACAACATCCTACAAATGTGAAACCCAGAACCCAGTGAGTGTCAGACGCAGCGACCCAGTCACCCTGAACGTCC

               (701) 701       710       720       730       740       750       760       770       780       790       800
Human CEACAM-5 (701) TCTATGGCCCGGATGCCCCCACCATTTCCCCTCTAAACACATCTTACAGATCAGGGGAAAATCTGAACCTCTCCTGCCACGCAGCCTCTAACCCACCTGC
Rhesus CEACAM-5 (701) TCTATGGCCCGGATGCGCCCACCATTTCCCCTCTAAACACACCTTACAGAGCAGGGGAAAATCTGAACCTCACCTGCCACGCAGCCTCTAACCCAACTGC
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FIG.6A

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Human CEACAM-5  (801) ACAGTACTCTTGGTTTGTCAATGGGACTTTCCAGCAATCCACCCAAGAGCTCTTTATCCCCAACATCACTGTGAATAATAGTGGATCCTATACGTGCCAA
Rhesus CEACAM-5 (801) ACAGTACTTTTGGTTTGTCAATGGGACGTTCCAGCAATCCACACAAGAGCTCTTTATACCCAACATCACCGTGAATAATAGCGGATCCTATATGTGCCAA

                (901)  901       910       920       930       940       950       960       970       980       990  1000
Human CEACAM-5  (901) GCCCATAACTCAGACACTGGCCTCAATAGGACCACAGTCACGACGATCACAGTCTATGCAGAGCCACCCAAACCCTTCATCACCAGCAACAACTCCAACC
Rhesus CEACAM-5 (901) GCCCATAACTCAGCCACTGGCCTCAATAGGACCACAGTCACGGCGATCACAGTCTACGCGGAGCTGCCCAAGCCCTACATCACCAGCAACAACTCCAACC

               (1001) 1001      1010      1020      1030      1040      1050      1060      1070      1080      1090 1100
Human CEACAM-5 (1001) CCGTGGAGGATGAGGATGCTGTAGCCTTAACCTGTGAACCTGAGATTCAGAACACAACCTACCTGTGGTGGGTAAATAATCAGAGCCTCCCGGTCAGTCC
Rhesus CEACAM-5 (1001) CCATAGAGGACAAGGATGCTGTGACCTTAACCTGTGAACCTGAGACTCAGGACACAACCTACCTGTGGTGGGTAAACAATCAGAGCCTCTCGGTCAGTTC

               (1101) 1101      1110      1120      1130      1140      1150      1160      1170      1180      1190 1200
Human CEACAM-5 (1101) CAGGCTGCAGCTGTCCAATGACAACAGGACCCTCACTCTACTCAGTGTCACAAGGAATGATGTAGGACCCTATGAGTGTGGAATCCAGAACGAATTAAGT
Rhesus CEACAM-5 (1101) CAGGCTGGAGCTGTCCAATGACAACAGGACCCTCACTGTATTCAATATTCCAAGAAACGACACAACGTTCTACGAATGTGAGACCCAGAACCCAGTGAGT

               (1201) 1201      1210      1220      1230      1240      1250      1260      1270      1280      1290 1300
Human CEACAM-5 (1201) GTTGACCACAGCGACCCAGTCATCCTGAATGTCCTCTATGGCCCAGACGACCCCACCATTTCCCCCTCATACACCTATTACCGTCCAGGGGTGAACCTCA
Rhesus CEACAM-5 (1201) GTCAGACGCAGCGACCCAGTCACCCTGAATGTCCTCTATGGCCCGGATGCGCCCACCATTTCCCCTCTAAACACACCTTACAGAGCAGGGGAAAATCTGA

               (1301) 1301      1310      1320      1330      1340      1350      1360      1370      1380      1390 1400
Human CEACAM-5 (1301) GCCTCTCCTGCCATGCAGCCTCTAACCCACCTGCACAGTATTCTTGGCTGATTGATGGGAACATCCAGCAACACACACAAGAGCTCTTTATCTCCAACAT
Rhesus CEACAM-5 (1301) ACCTCTCCTGCCACGCAGCCTCTAACCCAGCTGCACAGTACTCTTGGTTTGTCAATGGGACGTTCCAGCAATCCACACAAGAGCTCTTTATACCCAACAT

               (1401) 1401      1410      1420      1430      1440      1450      1460      1470      1480      1490 1500
Human CEACAM-5 (1401) CACTGAGAAGAACAGCGGACTCTATACCTGCCAGGCCAATAACTCAGCCAGTGGCCACAGCAGGACTACAGTCAAGACAATCACAGTCTCTGCGGAGCTG
Rhesus CEACAM-5 (1401) CACCGTGAATAATAGCGGATCCTATATGTGCCAAGCCCATAACTCAGCCACTGGCCTCAATAGGACCACAGTCACGGCGATCACAGTCTATGTGGAGCTG

               (1501) 1501      1510      1520      1530      1540      1550      1560      1570      1580      1590 1600
Human CEACAM-5 (1501) CCCAAGCCCTCCATCTCCAGCAACAACTCCAAACCCGTGGAGGACAAGGATGCTGTGGCCTTCACCTGTGAACCTGAGGCTCAGAACACAACCTACCTGT
Rhesus CEACAM-5 (1501) CCCAAGCCCTACATCTCCAGCAACAACTCCAACCCCATAGAGGACAAGGATGCTGTGACCTTAACCTGTGAACCTGTGGCTGAGAACACAACCTACCTGT
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FIG.6B

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Human CEACAM-5 (1601) GGTGGGTAAACAATCAGAGCCTCTCGGTCAGTCCCAGGCTGCAGCTCTCCAATGGCAACAGGATCCTCACTCTACTCAGTGTCACACGGAATGACACAGG
Rhesus CEACAM-5 (1601) GGTGGGTAAACAATCAGAGCCTCTCGGTCAGTCCCAGGCTGCAGCTCTCCAATGGCAACAGGATCCTCACTCTACTCAGTGTCACACGGAATGACACAGG

                (1701)  1701      1710      1720      1730      1740      1750      1760      1770      1780      1790 1800
Human CEACAM-5 (1701) AGCCTATGTATGTGGAATCCAGAACTCAGTGAGTGCAAACCGCAGTGACCAGTCACCCTGGATGTTCCTCTATGGGCCGGACACCCCCATCATTTCCCCC
Rhesus CEACAM-5 (1701) ACCCTATGAATGTGGAATCCAGAACTCAGAGAGTGCAAAACGCAGTGACCCAGTCACCCTGAATGTCACCTATGGCCCAGACACCCCCATCATATCCCCC

                (1801)  1801      1810      1820      1830      1840      1850      1860      1870      1880      1890 1900
Human CEACAM-5 (1801) CCAGACTCGTCTTACCTTTCGGGAGCGAACCTCAACCTCTCCTGCCACTCGGCCTCTAACCCATCCCCGCAGTATTCTTGGCGTATCAATGGGATACCGC
Rhesus CEACAM-5 (1801) CCAGACTTGTCTTACCGTTCGGGAGCAAACCTCAACCTCTCCTGCCACTCGGACTCTAACCCATCCCCGCAGTATTCTTGGCTTATCAATGGGACACTGC

                (1901)  1901      1910      1920      1930      1940      1950      1960      1970      1980      1990 2000
Human CEACAM-5 (1901) AGCAACACACACAAGTTCTCTTTATCGCCAAAATCACGCCAAATAATAACGGGACCTATGCCTGTTTTGTCTCTAACTTGGCTACTGGCCGCAATAATTC
Rhesus CEACAM-5 (1901) GGCAACACACACAAGTTCTCTTTATCTCCAAAATCACATCAAACAATAGCGGGGCCTATGCCTGTTTTGTCTCTAACTTGGCTACCGGTCGCAATAACTC

                (2001)  2001      2010      2020      2030      2040      2050      2060      2070      2080      2090 2100
Human CEACAM-5 (2001) CATAGTCAAGAGCATCACAGTCTCT----------GCATCTGGAACTTCTCCTGGTCTCTCAGCTGGGGCCACTGTCGGCATCATGATTGGAGTGCTG
Rhesus CEACAM-5 (2001) CATAGTCAAGAACATCTCAGTCTCCTCTGGCGATTCAGCACCTGGAAGTTCT---GGTCTCTCAGCTAGGGCTACTGTCGGCATCATAATTGGAATGCTG

                (2101)  2101      2110  2121
Human CEACAM-5 (2089) GTTGGGGTTGCTCTGATATAG (SEQ ID NO:6)
Rhesus CEACAM-5 (2098) GTTGGGGTTGCTCTGATGTAG (SEQ ID NO:1)
```

FIG.6C

```
                (1)  1        10        20        30        40        50        60        70        80        90    100
Human CEACAM-5  (1)  MESPSAPPHRWCIPWQRLLITASLLTFWNPPTTAKLTIESTPFNVAEGKEVLLLVHNLPQHLFGYSWWKGERVDGNRQIIGYVIGTQQATPGPAYSGREI
Rhesus CEACAM-5 (1)  MGSPSAPLHRWCIPWQRLLITASLLTFWNPPTTAQLTTESRPFNVAEGKEVLLLAHNVSQNLFGYIWYKGERVDASRRIGSCVIRTQQTTPGPAHSGRET

                (101)  101       110       120       130       140       150       160       170       180       190   200
Human CEACAM-5 (101)  IYPNASLLIQNIIQNDTGFYTLHVIKSDLVNEEATGQFRVYPELPKPSISSNNSKPVEDKDAVAFTCEPETQDATYDAWVNNQSLPVSPRLQLSNGNRTL
Rhesus CEACAM-5 (101)  IDFNASLLIHNVTQSDTGSYTIQVIKEDLVNEEATGQFRVYPELPKPYISSNNSNPVEDKDAVALTCEPETQDTTYLWWVNNQSLPVSPRLELSSDNRTL

                (201)  201       210       220       230       240       250       260       270       280       290   300
Human CEACAM-5 (201)  TLFNVTRNDTASYKCETQNPVSARRSDSVILNVLYGPDAPTISPLNTSYRSGENLNLSCHAASNPPAQYSWFVNGTFQQSTQELFIPNTTVNNSGSYTQQ
Rhesus CEACAM-5 (201)  TVFNIPRNDTTSYKCETQNPVSVRRSDPVTLNVLYGPDAPTISPLNTPYRAGENLNLTCHAASNPTAQYFWFVNGTFQQSTQELFIPNTTVNNSGSYMQQ

                (301)  301       310       320       330       340       350       360       370       380       390   400
Human CEACAM-5 (301)  AHNSDTGLNRTIVITITVYAEPPKPFITSNNSNPVEDEDAVALTCEPEIQNTTYLWWVNNQSLPVSPRLQLSNDNRTLTLLSVTRNDVGPYECGIQNELS
Rhesus CEACAM-5 (301)  AHNSATGLNRTIVIAITVYAELPKPYITSNNSNPIEDKDAVTLTCEPETQDTTYLWWVNNQSLSVSSRLELSNDNRTLTVFNIPRNDTTFYECETQNPVS

                (401)  401       410       420       430       440       450       460       470       480       490   500
Human CEACAM-5 (401)  VDHSDPVILNVLYGPDDPTISPSYTYYRPGVNLSLSCHAASNPPAQYSWLIDGNIQQHTQELFISNITEKNSGLYTQQANNSASGHSRTTVKTTIVSAEL
Rhesus CEACAM-5 (401)  VRRSDPVTLNVLYGPDAPTISPLNTPYRAGENLNLSCHAASNPAAQYSWFVNGTFQQSTQELFIPNTTVNNSGSYMQQAHNSATGLNRTTVTAITVYVEL

                (501)  501       510       520       530       540       550       560       570       580       590   600
Human CEACAM-5 (501)  PKPSISSNNSKPVEDKDAVAFTCEPEAQNITYLWWVNGQSLPVSPRLQLSNGNRTLTLFNVTRNDARAYVCGIQNSVSANRSDPVTLDVLYGPDTPIISP
Rhesus CEACAM-5 (501)  PKPYISSNNSNPIEDKDAVTLTCEPVAENITYLWWVNNQSLSVSPRLQLSNGNRILTLLSVIRNDTGPYECGIQNSESAKRSDPVTLNVTYGPDTPIISP

                (601)  601       610       620       630       640       650       660       670       680       690   700
Human CEACAM-5 (601)  PDSSYLSGANLNLSCHSASNPSPQYSWRINGIPQQHTQVLFIAKITPNNNGTYACFVSNLATGRNNSIVKSITVSASGTSPG---LSAGATVGIMIGVLV
Rhesus CEACAM-5 (601)  PDLSYRSGANLNLSCHSDSNPSPQYSWLINGTLRQHTQVLFISKITSNNSGAYACFVSNLATGRNNSIVKNISVSSGDSAPGSSGLSARATVGIIIGMLV

                (701)  706
Human CEACAM-5 (698)  GVALI (SEQ ID NO:7)
Rhesus CEACAM-5 (701)  GVALM (SEQ ID NO:2)
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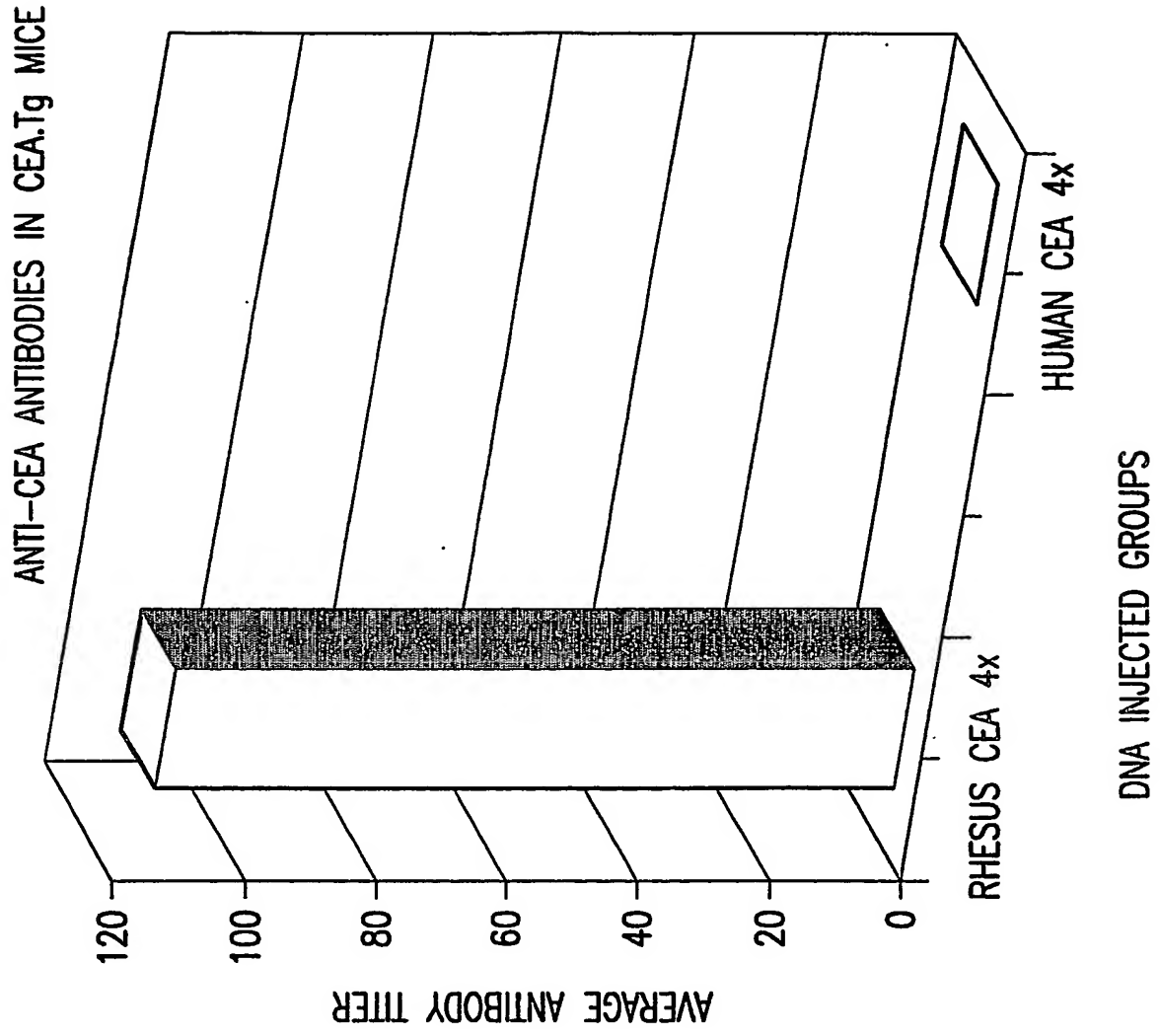
FIG.7

FIG.8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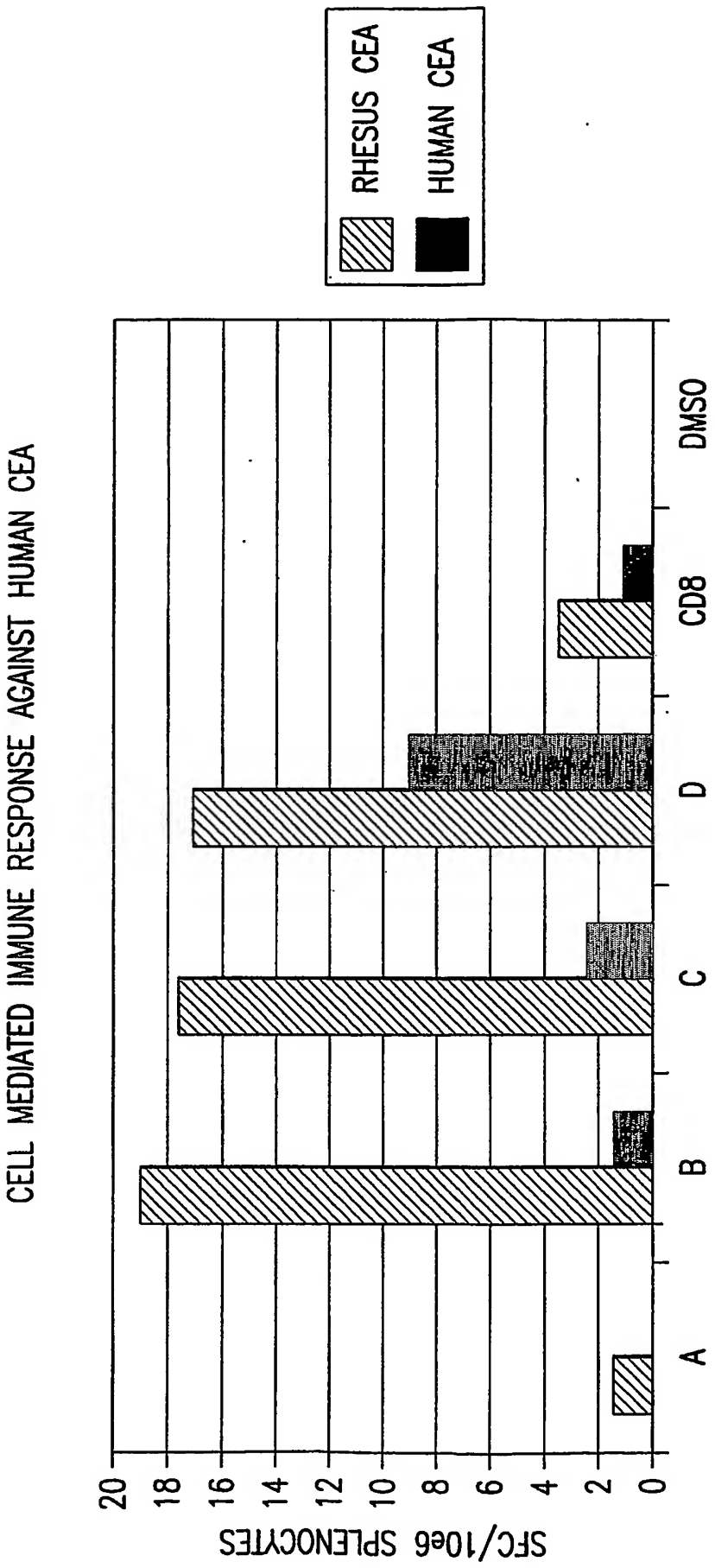
DNA INJECTED GROUPS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HUMAN CEA
SFC/10e6 SPLENOCYTES
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2
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20
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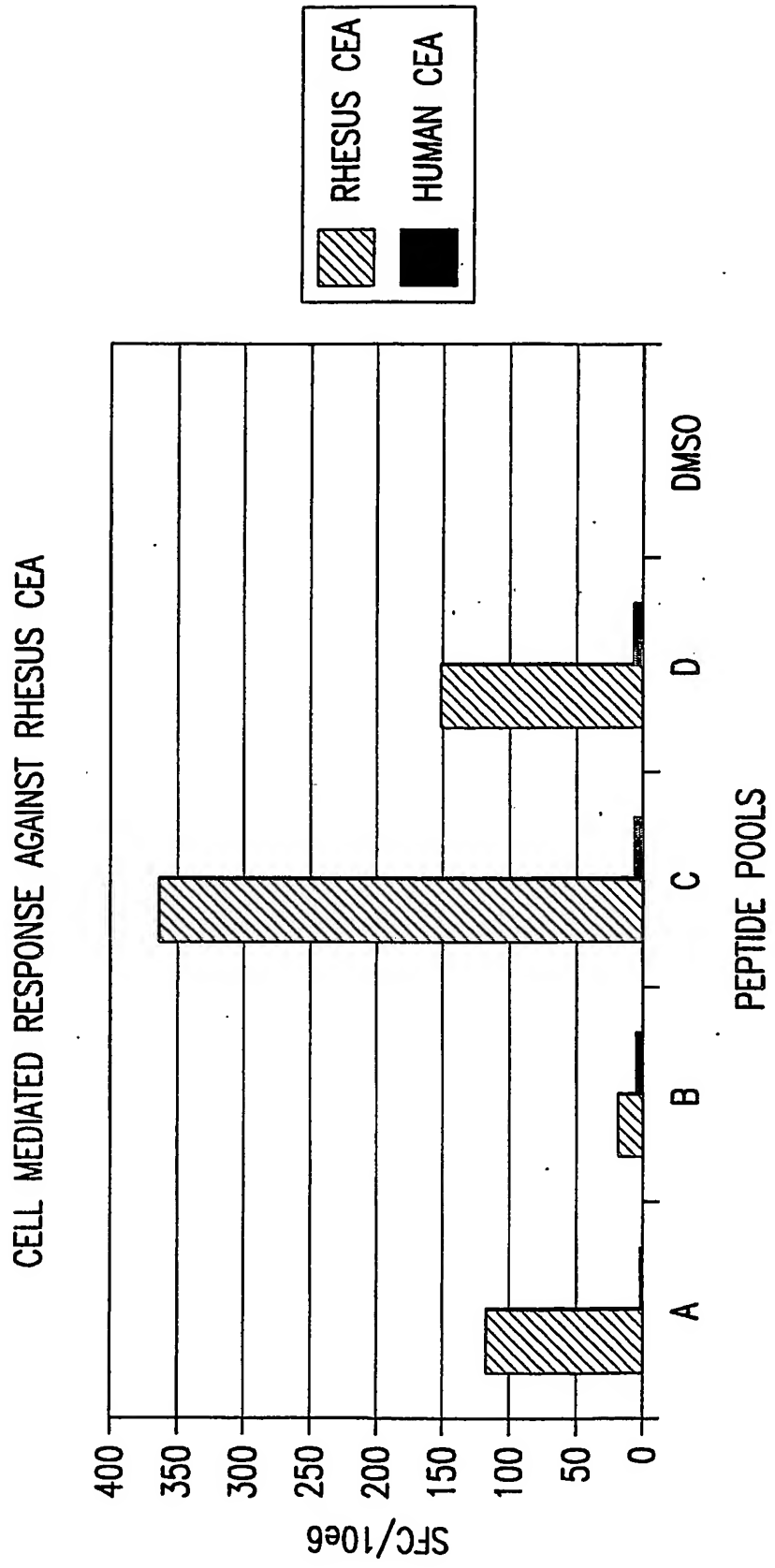
B
C
D
CD8
DMSO
PEPTIDE POOLS

FIG.9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HUMAN CEA
CELL MEDIATED RESPONSE AGAINST RHESUS CEA
SFC/10e6
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250
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100
50
0
A
B
C
D
DMSO
PEPTIDE POOLS

FIG.10

SEQUENCE LISTING

110> Luigi Aurisicchio
Fabio Palombo
Paolo Monaci
Nicola La Monica
Gennaro Ciliberto
Armin Lahm

120> RHESUS CARCINO EMBRYONIC ANTIGEN, NUCLEOTIDES ENCODING SAME, AND USES THEREOF

130> ITR0045 PCT

150> 60/447,203
151> 2003-02-13

160> 16

170> FastSEQ for Windows Version 4.0

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212> DNA
213> Macaca mulatta

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Ser Ala Glu Leu Pro Lys Pro Ser Ile Ser Ser Asn Asn Ser Lys Pro

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/001181

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/63 C12N15/861 C12N1/12 C07K14/705 C12N5/10
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBASE, BIOSIS, CHEM ABS Data, EPO-Internal, WPI Data, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 274 087 A (KAMARCK MICHAEL E ET AL) 28 December 1993 (1993-12-28) cited in the application column 20, line 55 -column 21, line 68 --- -/--	1-34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2004	06/08/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Barnas, C

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WHEELER C W ET AL: "Safety and immunogenicity of a recombinant vaccinia virus vaccine expressing a 70kDa fragment of the human carcinoembryonic antigen in a nonhuman primate" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 36, no. 0, 1995, page 366 XP001181916 Eighty-sixth Annual Meeting of the American Association for Cancer Research;Toronto, Ontario, Canada; March 18-22, 1995, 1995 ISSN: 0197-016X abstract	1-34
A	IRVINE K ET AL: "CHARACTERIZATION OF IMMUNOLOGICAL RESPONSES USING A CEA RECOMBINANT VACCINIA VIRUS IN NON-HUMAN PRIMATES" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 33, 1992, page 334 XP001181917 83RD ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN DIEGO, CALIFORNIA, USA, MAY, 1992 ISSN: 0197-016X abstract	1-34
A	KANTOR J ET AL: "Immunogenicity and safety of a recombinant vaccinia virus vaccine expressing the carcinoembryonic antigen gene in a nonhuman primate" CANCER RESEARCH, vol. 52, no. 24, 1992, pages 6917-6925, XP000605381 ISSN: 0008-5472 abstract page 6920, left-hand column, paragraph 2 -page 6923, right-hand column, paragraph 1	1-34
A	JANTSCHEFF P ET AL: "SEARCH FOR CARCINOEMBRYONIC ANTIGEN-LIKE MOLECULES IN POLYMORPHONUCLEAR LEUKOCYTES OF NON-HUMAN PRIMATES USING MONOCLONAL ANTIBODIES" ARCHIV FUER GESCHWULSTFORSCHUNG, vol. 56, no. 2, 1986, pages 113-116, XP001181929 ISSN: 0003-911X abstract page 8, paragraph 1	1-34

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/001181

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/30382 A (AVENTIS PASTEUR ;BARBER BRIAN (CA); BERINSTEIN NEIL (CA); MOINGEON) 3 May 2001 (2001-05-03) page 2, line 12 -page 3, line 3 example 1 claims 1,2,4,5	1-34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/001181

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 20-24, 29-34
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 20-24, 29-34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.1

Claims Nos.: 20-24, 29-34

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/001181

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   - [X] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material

   - [X] in written format
   - [X] in computer readable form

   c. time of filing/furnishing

   - [X] contained in the international application as filed
   - [ ] filed together with the international application in computer readable form
   - [ ] furnished subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

International Application No
PCT/EP2004/001181

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5274087 A	28-12-1993	US 5571710 A	05-11-1996
		US 5843761 A	01-12-1998
		AT 96168 T	15-11-1993
		AU 598573 B2	28-06-1990
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		FI 873478 A	14-02-1988
		IE 60279 B1	29-06-1994
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		CA 2388337 A1	03-05-2001
		EP 1227837 A1	07-08-2002
		EP 1228095 A1	07-08-2002
		JP 2003512437 T	02-04-2003
		JP 2003512829 T	08-04-2003